# COMPARITIVE EVALUATION OF THE REMINERALIZING POTENTIAL OF THREE DIFFERENT DENTIFRICES – AN *IN VITRO* STUDY EVALUATED USING MICRO CT AND MICROHARDNESS TESTING

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**DEPARTMENT OF CONSERVATIVE DENTISTRY & ENDODONTICS**

# *CERTIFICATE*

This is to certify that this dissertation entitled **"COMPARITIVE EVALUATION OF THE REMINERALIZING POTENTIAL OF THREE DIFFERENT DENTIFRICES – AN *IN VITRO* STUDY EVALUATED USING MICRO CT AND MICROHARDNESS TESTING"** is a genuine work done by **Dr. Arun Balakrishnan** under my guidance during his post graduate study period between 2010-2013.

This Dissertation is submitted to **THE TAMILNADU Dr. M.G.R. Medical University**, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY IN CONSERVATIVE DENTISTRY AND ENDODONTICS BRANCH IV.** It has not been submitted (partial or full) for the award of any other degree or diploma.

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Place Name

Date Sign

## LIST OF ABBREVATIONS USED

| ABBREVATIONS | WORD EXPLANATION |
|---|---|
| CPP – ACP | Casein Phosphopeptide – Amorphous Calcium Phosphate |
| CSP | Calcium sodium silicophosphate |
| f-TCP | Functionalized Tricalcium phosphate |
| $F^-$ | Fluoride |
| FAP | Fluorapatite |
| HAP | Hydroxyapatite |
| HCA | Hydroxyl carbonate Apatite |
| LAC | Linear Attenuation Co-efficient |
| µ-CT | Microcomputed Tomography |
| MID | Minimal invasive dentistry |
| NaF | Sodium Fluoride |
| SLS | Sodium lauryl sulphate |
| SMH | Surface Microhardness |
| VHN | Vickers hardness number |
| ANOVA | Analysis Of Variance |
| P – value | Probability value |
| F - value | Fischer’s value |

# INTRODUCTION

*Caries* is not just a disease, but a disease process[1]. The current concept considers caries as a dynamic and reversible process which is the result of the interplay of numerous etiological factors[2]. Some of these factors cause demineralization whereas others promote re-mineralization of the tooth. When demineralization process continues, it leads to cavitation. Caries can be arrested or even reversed at the pre–cavitated stage, provided a balance of re-mineralization is established[2].

The term *"Minimal Invasive Dentistry"* (MID) can be best defined as the management of caries with a biological approach, rather than with a traditional operative approach. The goal of MID is to first stop the disease process and then to restore lost tooth structure and function, maximizing the healing potential of the tooth[3]. MID focuses on the least invasive treatment options possible in order to minimize tissue loss and patient discomfort. Concentrating mainly on prevention and early intervention of caries, MID's first basic principle is the remineralization of early carious lesions, advocating a biological or therapeutic approach rather than the traditional surgical approach for early surface lesions. One of the key elements of a biological approach is the usage and application of remineralizing agents to tooth structure. These agents are part of a new era of dentistry aimed at controlling the demineralization/ remineralization cycle, depending upon the microenvironment around the tooth[4].

*Demineralization* is the process of removing minerals, in the form of mineral ions, from tooth enamel[4,5]. A substantial number of mineral ions can be removed from hydroxyapatite latticework without destroying its structural integrity. When too many minerals are dissolved from an area of the hydroxyapatite's latticework, it results in a cavity that is the loss of the hydroxyapatite's crystalline latticework structure. The latticework can be strengthened and restored through the process of remineralization.

*Remineralization* is the process of restoring minerals in the form of mineral ions to the hydroxyapatite latticework structure. Remineralization should be three-dimensional and must be replaced with same shape, size and the same electrical charge as those lost from the lattice.

In last decade, various remineralizing agents have been introduced, most of which contain fluoride, calcium, and phosphate ions in varied forms and concentrations. These agents tend to remineralize the subsurface caries lesion by providing calcium phosphate with or without fluoride and control the surrounding micro-environment.

The concept of Casein Phosphopeptide - Amorphous Calcium Phosphate (CPP - ACP) as a remineralizing agent was first postulated in 1998[6]. CPP - ACP nanocomplexes are derived from bovine milk protein; casein, calcium and

phosphate. Tooth mousse (GC International, Itabashi - Ku, Tokyo, Japan) contains nanocomplexes of milk protein Casein Phosphopeptide (CPP) with Amorphous Calciumphosphate (ACP). It has been suggested that CPP has the ability to stabilize calcium phosphate in solution by binding ACP with their multiple phosphoserine residues, thereby allowing formation of small CPP - ACP clusters. CPP containing the sequence Ser (P) - Ser (P) - Ser (P) - Glu – Glu stabilized nanoclusters of ACP in metastable solution[7].

The multiple phosphoseryl residues of the CPP bind to form nanoclusters of ACP in supersaturated solutions, that prevent growth to the critical size required for phase transformations. It has been claimed that it promotes remineralization of the carious lesions by maintaining a supersaturated state of enamel minerals, at the same time it also hinders colonization of dental surfaces by cariogenic bacteria. The proposed anticariogenic mechanism of CPP - ACP is the incorporation of nanocomplexes into plaque and onto tooth surfaces[7].

Clinpro™ tooth crème (3M) is a 0.21% w/w Sodium Fluoride Anti Cavity Paste that contains 950 ppm fluoride and a functionalized tri-calcium phosphate ingredient (f-TCP). The novel functionalized tricalcium phosphate is prepared by reacting sparingly soluble Tricalcium Phosphate (TCP) with surfactant (Sodium Lauryl Sulphate). One main feature of this calcium phosphate system is that it is

stable in aqueous environment and also does not affect the fluoride activity added in the dentifrices[8]. Also it has been suggested that fluoride combination with functionalised Tricalcium Phosphate (f-TCP) not only provides greater remineralization in terms of microhardness and fluoride uptake, but also decreases the dose of fluoride required to achieve the same degree of remineralization. Enamel fluoride uptake is improved in the presence of the functionalized calcium phosphate, as this agent coordinates with fluoride to help promote mineral nucleation in weakened enamel[9].

Novamin containing SHY - NM (Group Pharnaceuticals) is a bioactive glass in the class of highly biocompatible materials that were originally developed as bone regenerative materials[10]. These materials are reactive when exposed to body fluids and deposit Hydroxy Carbonate Apatite (HCA), a mineral that is chemically similar to natural tooth minerals. Shy - NM is a dentifrice containing Calcium sodium phosphosilicate (Novamin). In aqueous environments such as saliva, sodium ions in calcium sodium phosphosilicate particles immediately (within one minute) begin to exchange with hydrogen cations. This rapid exchange of ions allow calcium and phosphate species to be released from the particle structure. A modest localized, transient increase in pH occurs that facilitates the precipitation of calcium and phosphate from the particles and from saliva to form a calcium phosphate (Ca - P) layer on tooth surfaces. As the

reactions and the deposition of the Ca - P complexes continue this layer crystallizes into (HCA), which is chemically and structurally similar to biologic apatite[11].

Micro-focus X-ray CT (micro-CT) analysis has made it possible to describe a serial mineral change during a demineralization and remineralization non - destructively. The non - destructive approach of micro CT makes it possible to study the mineral concentration more accurately whilst overcoming the shortcomings of earlier destructive study techniques. Another advantage of this method is that the mineral content of teeth can be reconstructed and observed from various angles.

Considering the importance of the surface layer in caries progression, the evaluation of changes in this region is relevant. Surface Micro Hardness (SMH) measurement was a suitable technique for this purpose. Micro hardness measurement was appropriate for a material having fine microstructure, non-homogenous or prone to cracking like enamel. SMH indentations provide a relatively simple, non-destructive and rapid method in demineralization and remineralization studies.

All the three remineralizing agents mentioned above, differ in their composition and mechanism of action, yet each one has a promising ability to remineralize the enamel. Therefore this study was undertaken to determine and compare the microhardness values as well as the remineralizing potential of CPP - ACP (GC Tooth Mousse), 0.21% NaF with functionalized tricalcium phosphate fTCP (Clinpro) and bioactive glass Novamin (SHY - NM).

# AIMS AND OBJECTIVES

1. To evaluate remineralization potential of artificial enamel carious lesion using Casein Phosphopeptide - Amorphous Calcium Phosphate (GC Tooth Mousse).

2. To evaluate remineralization potential of artificial enamel carious lesion using 0.21% sodium fluoride - tricalcium phosphate (Clinpro tooth crème).

3. To evaluate remineralization potential of artificial enamel carious lesion using bioactive glass containing dentifrice (SHY - NM).

4. To do a comparative evaluation to determine the remineralization potential of Casein Phosphopeptide - Amorphous Calcium Phosphate, 0.21% sodium fluoride - tricalcium phosphate, bioactive glass containing dentifrice.

# REVIEW OF LITERATURE

In **1987, E.C Reynolds**[12] conducted a study to determine the ability of bovine milk phosphoprotein (casein) to prevent enamel sub surface demineralization and affect the bacterial composition using a modified intraoral caries model. The model consisted of a removable appliance containing a right and left pair of bovine enamel slabs. Supragingival plaque was then collected and incorporated over these slabs. Left side of the appliance was exposed to various sugar and salt solutions while the right side was exposed to sugar and casein solution. The results showed that the ability of casein and tryptic peptides to prevent enamel demineralization which was in turn related to their incorporation into plaque and thereby increasing plaque calcium phosphate and acid buffering capacity by the phosphoseryl, histidyl, glutamyl, and aspartyl residues and indirectly through catabolism by plaque bacteria.

**Featherstone et al** in **1990**[13] conducted a study to evaluate the effect of fluoride concentration on demineralization remineralization of apatite of dental enamel and results of his study suggested that simply increasing fluoride concentration may not necessarily give increased cariostatic benefit, and that improving the means of delivery of relatively low fluoride concentrations for longer times should be more appropriate for enhancing clinical efficacy.

In **1994, White et al**[14] showed that fluorohydroxyapatite formation represents the equilibrium mineral phase composition of many fluoridated biological minerals, in

agreement with theoretical predictions. However it is expected that caries protection requires constant resupply of fluoride reactivity and that caries benefits are at least partially lost without continuous topical application. The development of absolute resistance to caries in the teeth through FAP formation is a myth. Thus a daily exposure to trace concentrations of fluoride in the form of dentifrice or mouthwash is essential for caries prevention

**E.C Reynolds et al**[15] in **1995** conducted a study to investigate the ability of CPP - ACP to reduce caries activity by use of specific pathogen free rats inoculated with streptococcus sobrinus. The results of the study showed that CPP - ACP significantly reduced caries activity in a dose-response fashion, with 1.0% CPP - ACP producing 55% and 46% reductions in smooth surface and fissure caries activity respectively being similar to that of 500 ppm F, The synthetic octapeptide - calcium phosphate complex significantly reduced caries activity. They concluded from their study that the anticariogenic effects of CPP - ACP and fluoride were additive, since animals receiving 0.5% CPP - ACP plus 500 ppm F had significantly lower caries activity than those animals receiving either CPP - ACP or fluoride alone.

**Reynolds EC** in **1997**[16] conducted a study to examine the effects of CPP - stabilized calcium concentration. The study comprised of sound, ground buccal and lingual surfaces of extracted human teeth. Artificial carious lesions were created and solutions were then

used to examine the effect of CPP - ACP concentration on remineralization. After a period of 10 days remineralized enamel lesions were sectioned, subjected to microradiography and the mineral content determined by micro densitometry. It was then seen that the remineralization potential was greater for the solutions with the higher levels of CPP - stabilized free calcium and phosphate ions and the remineralization does not significantly correlate with either CPP bound ACP or the degree of saturation.

**T. Attin, A.M Kielbassa, M. Schwanenberg, E.Hellwig** in **1997**[17] conducted a study to evaluate the remineralizing capacity of different fluoride treatments on dental enamel bleached with carbamide peroxide. 60 enamel specimens were subjected to four cycles comprising bleaching (12h) and remineralization in artificial saliva (8h). It was concluded that remineralization of bleached enamel is improved by application of highly concentrated fluorides.

**Eric C Reynolds** in **1998**[18] conducted a study in laboratory animals and human in situ caries models to show that CPP - ACP complexes exhibit an anticariogenic activity. The study demonstrated that CPP - ACP localize ACP in dental plaque, which buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel depressing demineralization and enhancing remineralization. The CPP - ACP, unlike fluoride can be added to sugar containing

foods and therefore have commercial potential as an additive to foods, toothpastes and mouthwashes for the control of dental caries.

An in vitro study was conducted by **S. Takagi, H.Liao, L.C Chow**[19] in **2003** to determine the efficacy of tooth bound fluoride on enamel demineralization or remineralization. In this study, the enamel sections were prepared and placed in remineralization solution for a period of five days and were then randomly grouped into three groups. Group I; the control group which received no treatment. Group II; received APF and group III received DCPD - APF. After the treatment, sections were washed in constant composition F titration system to remove loosely bound F. The subjects were then exposed to cycles of demineralization and remineralization to produce caries like lesions. The results showed that enamel resistance to lesion formation increased with increasing tooth bound fluoride.

**E.C Reynolds, F. Cai, P. Shen and G.D Walker**[20] in **2003** conducted a study to compare the ability of CPP - ACP, with other forms of calcium to remineralize enamel subsurface lesions and retain it in supragingival plaque when delivered in a mouthrinse or a sugar free gum. The results showed that in the mouth rinse groups, only CPP - ACP containing mouth rinse significantly increased plaque calcium and inorganic phosphate levels. In the chewing gum groups, the gum containing CPP - ACP produced highest levels of enamel remineralization independent of gum chewing frequency and duration.

With the above observations they concluded that CPP - ACP was superior to other forms of calcium in remineralizing enamel sub surface lesion and this study highlights the important role of the CPP as an ACP carrier localizing the highly soluble calcium phosphate phase at the tooth surface.

In **2003, S.A Mazzaoui, M.F. Burrow, M.J Tyas, S.G Dashper, D. Eakins and E.C Reynolds**[21] conducted a study to determine the effect of incorporating CPP - ACP into self cured glass ionomer cement. Incorporation of 1.56% w/w CPP - ACP into the GIC resulted in significant increase in the microtensile bond strength (33%) and compressive strength (23%) and also significantly enhanced the release of calcium, phosphate and fluoride ions at neutral and acidic pH. MALDI mass spectrometry also showed CPP from the CPP - ACP nanocomplexes to be released. They concluded from their study that the release of CPP - ACP and fluoride from the CPP - ACP containing GIC was associated with enhanced protection of the adjacent dentin during acid challenge.

In **2003, F Cai, P Shen, MV Morgan, EC Reynolds**[22] conducted a study to determine the effect of CPP - ACP incorporation into a sugar free lozenge on enamel remineralization in a human in situ model. The study utilized four treatments: (i) a lozenge containing 56.4 mg (3 percent w/w) CPP - ACP; (ii) a lozenge containing 18.8 mg (1 percent w/w) CPP - ACP; (iii) a lozenge not containing CPP - ACP and (iv) a no lozenge nil- treatment control. Ten subjects wore removable palatal appliances

with four human enamel, half - slabs insets containing subsurface lesions. The results of the study showed that incorporation of CPP - ACP into the lozenge significantly increased enamel subsurface lesion remineralization. They concluded from their study that lozenges are a suitable vehicle for the delivery of CPP - ACP to promote enamel remineralization.

An **in vitro study was conducted by Mithra N Hegde, Shishir shetty, Deepak Pardal in 2007**[23] to evaluate the remineralization potential of CPP - ACP paste on artificial enamel sub surface lesion using EDAX. Their study comprised of 60 specimens which were evaluated for the mineral content using EDAX. The specimens were placed in demineralizing solution for 48 hours to produce artificial carious like lesion and again it was subjected to EDAX and were randomly assigned into three groups and one control group and the procedures were carried out. Their results showed that the study groups showed an increase in mineral content as compared to demineralized samples and no change was seen in control group. They concluded that 10% CPP - ACP was significant enough to remineralize the artificial enamel sub-surface lesion.

**Jeremy Rees, Theresa Loyn, Barbara Chadwick**[24] in **2007** did a study to examine whether a single topical application of proenamel or tooth mousse would prevent enamel erosion. Enamel samples were treated with either proenamel or tooth mousse applied for 15 min. The control group was placed in distilled water for 15 min. All specimens were

then exposed to an erosive challenge of 0.2% citric acid for 1 h. From their study they concluded that Tooth mousse and proenamel may offer a degree of protection from erosion of permanent enamel.

**Maki oshiro et al** in **2007**[25] conducted a study to evaluate the effect of CPP - ACP paste on demineralization by observing the treated tooth surface using FE-SEM. In this study the specimens were prepared by cutting enamel and dentin of bovine teeth into blocks. In group I, the specimens were stored in 0.1 M lactic acid buffer solution for 10 minutes and then in artificial saliva which serves as the negative control and in the other group specimens were stored in a 10 times diluted solution of CPP - ACP paste or a placebo paste containing no CPP - ACP for 10 minutes. The results were evaluated using SEM. SEM observations revealed that demineralization of enamel and dentin surfaces was more pronounced with longer test period in the control and negative control period. On the other hand enamel and dentin specimens treated with CPP - ACP paste revealed slight change in morphologic features.

**Christos Rahiotis, George Vougiouklakis, George Eliades** in **2007**[26] conducted a study to compare the morphological appearance and the the molecular composition of intraoral integuments formed in situ on germanium (Ge) crystals in the presence or absence of the commercially available CPP - ACP cream agent. Six volunteers participated in the study. Impression of maxillary arch was taken for each patient, and a removable orthodontic

appliance with a custom-made retainer was fabricated. Clean Ge crystals mounted in the retainers were placed intraorally for 30 min, 8, 24 hours and 1 week period. The free sampling surface of another series of Ge crystals was treated with the commercial CPP - ACP agent (Tooth Mousse), mounted in the retainers and placed intraorally for the same period as above. The free exposed surfaces in the oral cavity of the specimens in all subjects were examined and they concluded that the presence of CPP - ACP agent delays the biofilm formation and favoured the nucleation and crystallization of calcium phosphates**,** possibly in apatitic form, in matured biofilms.

In **2008, F. Cai, N.J Cochrane, F.Shen, G.D Walker, M.V Morgan and E.C Reynolds**[27] conducted a study to determine the ability of CPP - ACP to increase the incorporation of fluoride into plaque and to promote enamel remineralization. A randomized, double blinded, cross over study involved the use of mouthrinses and dentifrices containing CPP - ACP and fluoride. The result showed that the addition of 2% CPP - ACP to the 450 ppm Fluoride mouthrinse significantly increased the incorporation of fluoride into plaque. Also the dentifrice containing 2% CPP - ACP produced a level of remineralization similar to that achieved with a dentifrice containing 2800 ppm F, though the dentifrice containing 2% CPP - ACP plus 1100 ppm F was superior to all other formulations.

An in vitro study was conducted by **H. Fujikawa, K. Matsuyama, A. Uchiyama, S. Nakashima, T. Ujiie** in **2008**[28] to investigate the influence of salivary macromolecules on enamel lesion remineralization in the presence or absence of fluoride. Artificial subsurface lesions were created on human enamel specimens at pH 4.5 acetate buffer. Group C had mineral solution as a control; Group S had mineral solution + dialysed saliva; Group F had mineral solution + 1ppm fluoride; Group SF had mineral solution = dialysed saliva + 1 ppm F. The results of the study showed a statistically significant mineral gain in the lesions in group C and SF, but not in group S and F. The study was concluded by explaining that the macromolecules inhibited lesion remineralization, but at the same time these molecules in the presence of fluoride play an important role in the remineralization by reducing mineral gain at the surface.

In **2008, M.J Altenburger, J.F Schirrmeister, K.T Wrbas, M. Klasser, E. Hellwig**[29] conducted a study to test the hypothesis that weekly application of a 1.25% fluoride gel results in significant higher fluoride uptake and mineral gain in comparison to 0.5% fluoride or a placebo gel, respectively. Thirty six subjects were included and randomly assigned to groups. Specimen holders each containing 10 bovine enamel slabs were placed in the subject's mouths for a period of three to four weeks. During the experimental period the volunteers brushed the specimen with placebo gel, 0.5% fluoride gel or 1.25% fluoride gel once a week. The results showed that a significant increase in fluoride uptake with 1.25% fluoride gel than with 0.5% fluoride gel or placebo gel,

whereas the changes in the mineral gain and lesion depth were not statistically significant between the three groups. They concluded from their study that repeated application of highly concentrated fluoride gel did not promote remineralization significantly.

In **2008, M.V Morgan, G.G Adams, D.L Bailey, C.E Tsao, S.L Fischman, E.C Reynolds**[30] conducted a study using digital bitewing radiography, to determine the progression and regression of approximal caries in adolescents chewing a sugar free gum containing 54 mg CPP - ACP relative to the identical chewing gum without CPP - ACP. For the study 2720 subjects were selected from 29 schools and were assigned randomly one of the two gums. They were instructed to chew their gums 3 times for 10 minutes per day, with one session supervised on school days over a period of 24 months. The results showed statistically significant difference in the frequency distributions of the transition scores between the two groups. It was concluded that 54 mg CPP - ACP sugar free gum significantly slowed the progression and enhanced regression of approximal caries in comparison to sugar free gum in a 24 month clinical trial.

An in vitro study was conducted by **MT Pulido, JS Wefel, MM Hernandez GE Denehy, S Guzman-Armstrong, JM Chalmers, F Qian** in **2008**[31] to evaluate the inhibition of demineralization in enamel sections produced by MI paste, fluoride and a combination of both, compared to artificial saliva and NaF 5000 ppm in a caries progression pH - cycling model and they concluded that, the higher concentration of NaF

(5000 ppm) reduced lesion progression to the greatest extent. The MI paste group did not show any effect on the inhibition of lesion progression.

In **2008, N.J Cochrane, S. Saranathan, F.Cai, K.J cross, E.C Reynolds**[32] conducted an in vitro study to determine the effect of ion composition of CPP - ACP and amorphous calcium fluoride phosphate (CPP - ACPF) on enamel sub surface remineralization. CPP bound and free calcium, phosphate and fluoride ion concentrations in the solutions were demineralized by ultrafiltration. The results showed that the remineralization of the subsurface occurred at all pH's (7.0 - 4.5) with maximum activity seen at pH 5.5 The CPP - ACFP solutions produced greater remineralization than the CPP - ACP solutions at pH 5.5 or below. The study concluded by stating that the mineral formed in the subsurface lesions was consistent with hydroxyapatite and fluorapatite for remineralization with CPP - ACP and CPP - ACFP, respectively.

In **2008, J.M. ten Cate, M.J. Buijs, C. Chaussain Miller, and R.A.M. Exterkate** [33] did a study to investigate the dose response between 0 and 5000 ppm F of de and remineralization of advanced enamel lesions. Treatments included sodium and amine fluoride, and a fluoride-free control. Treatments with 5000 ppm F both significantly enhanced remineralization and inhibited demineralization when compared with treatments with 1500 ppm F. They concluded that with 5000 – ppm F treatments, more demineralizing episodes would still be repaired by remineralization.

**J.F. Schirrmeister, R.K. Seger, M.J. Altenburger, A. Lussi, E. Hellwig** in **2008**[34] conducted a study to determine the effects of 4 chewing gums on artificial caries - like subsurface lesions. Two chewing gums (1) with zinc citrate and 1 without contained dicalcium phosphate (3.9%), calcium gluconate (1.8%) and calcium lactate (0.45%), (2) chewing gum contained CPP - ACP nanocomplexes (0.7%), and another one contained no calcium. Fifteen subjects without current caries activity wore removable buccal appliances in the lower jaw with 4 bovine enamel slabs with subsurface lesions. The appliances were inserted immediately before gum chewing for 20 minutes and then retained for an additional 20 min which was performed 4 times per day. Every subject chewed 4 different chewing gums over 4 periods of 14 days each. During a fifth period (control) the subjects only wore the appliances without chewing gum. From their study they concluded that the use of chewing gum offers no additional remineralizing benefit to buccal tooth surfaces, even if the chewing gum contains calcium compounds.

An in vitro study was conducted by **H.E Darshan, N.D Shashikiran** in **2008**[35] conducted an in vitro study to evaluate the effect of McInnes bleaching agent on the microhardness of enamel before and after bleaching and to evaluate the effect of G.C Tooth mousse on the bleached enamel surface for its microhardness. Here 10 freshly extracted teeth were selected which were then cut sagitally using diamond disc and the buccal surface were impregnated in the cold cure resin facing upwards which were then

made into pellets. They were then subjected to baseline microhardness testing, microhardness testing after application of McInnes solution and microhardness testing after application of GC Tooth mousse. They concluded that McInnes bleaching agent does decrease the microhardness of enamel by causing enamel demineralization and GC tooth mousse causes an increase in the microhardness of bleached enamel by maintaining a high gradient of calcium and phosphate ions at the enamel subsurface.

In **2009, Burwell A et al[36],** conducted a study to evaluate the remineralization potential of Novamin and protection against demineralization. The results of the study suggested that analysis of surface microhardness with a Novamin containing dentifrice creates a tenacious surface layer that protects dentin from demineralization caused by repeated acidic and mechanical challenges whereas CPP - ACP did not provide the same protection. Novamin improved hardening of white-spot lesions. Fluoride alone (1000-5000) ppm did not effectively repair demineralized dentin. However Novamin containing dentifrices re-hardened and, theoretically repaired the lesions

In **2009, F.C. Rehder Neto, F.A. Maeda, C.P. Turssi, M.C. Serra**[37] conducted a study to assess whether pastes containing CPP – ACP and CSP control artificial caries lesion progression. Incipient caries-like lesions were pre-formed and specimens were evaluated by microhardness test and randomly assigned to five treatment groups (n = 15): (1) regular dentifrice (RE, 1,100 ppm F); (2) dentifrice with calcium sodium

phosphosilicate (CSP); (3) amorphous calcium phosphate stabilized by casein phosphopeptide (CPP – ACP); (4) CPP – ACP with 900 ppm F (CPP – ACP + F) and (5) control group - unexposed to any remineralizing agent and treatments were applied five times, after the de-remineralization period in the cariogenic challenges and they concluded from their study that depending on the agent used, a remineralizing effect may be expected, which reflects in caries lesions progression.

An in vivo study was conducted by **S.K. Rao, G.S. Bhat, S. Aradhya, A. Devi, M. Bhat** in **2009**[38] conducted a study to evaluate the efficacy of CPP - containing toothpaste in preventing dental caries in school children. The study was conducted among 150 school children randomly divided into three groups, each using one of three types of toothpastes: (a) containing 2% w/w CPP; (b) containing 1,190 mg/kg fluoride as 0.76% sodium monofluorophosphate (SMFP); (c) placebo toothpaste without CPP or fluoride. Students brushed with the given toothpastes for 24 months. Oral hygiene and caries experience were assessed at baseline, 12 and 24 months. They concluded that CPP can be effectively incorporated into calcium carbonate-based toothpaste and that toothpaste containing CPP is effective in preventing caries. Toothpaste containing 2% CPP seemed to have an efficacy similar to paste containing 1,190 mg/kg SMFP in the prevention of caries.

An in vitro study was done by Alessandri **Bonetti Giulio, Zanarini Matteo, Incerti Parenti Serena, Marchionni Silvia and Checchi Luigi** in **2009**[39] to qualitatively evaluate the effect of Casein Phosphopeptide – Amorphous Calcium Phosphate on stripped enamel morphology after exposure to an acid solution, by means of scanning electron microscopy. For the study 15 lower incisors were extracted and underwent metal stripping by a single operator. One group served as control and the other to which tooth mousse containing CPP - ACP was tested. The results showed that in control group, stripped samples exhibited greater demineralization compared to unstripped. While in experimental group, (CPP - ACP) reduced enamel dissolution on both intact and abraded samples was seen. The authors concluded from their study that topical application of CPP - ACP could be effective in promoting enamel remineralization after interdental stripping.

**Burwell A, Jennings D, Muscle D, Greenspan DC** in **2010**[40] conducted a study to determine the ability of a Calcium Sodium Phosphosilicate (Novamin) particulate to occlude dentin tubules and to characterize the nature of the occlusion through a number of in vitro studies. The results suggested that Novamin based dentifrices release less calcium initially compared to the other treatment groups. After four hours, a higher release of calcium was observed that was sustained over 24 hours. Novamin adheres to an exposed dentin surface and reacts with it to form a mineralized layer. The layer formed is resistant to acid challenges and is mechanically strong. The continuous release of calcium

over time was suggested to maintain the protective effects on dentin, and provide continuous occlusion of dentinal tubules.

An in vitro study was conducted by **Raghuwar D Singh, Sabita M Ram, Omkar Shetty, Pooran Chand, Rakesh yadav** in **2010**[41] to show the efficacy of CPP - ACP to prevent stain absorption on freshly bleached enamel. In their study forty extracted human maxillary central incisors were subjected to bleaching with 10% carbamide peroxide for eight days which were then divided into four groups of 10 each. Group I as control, Group II immersed in tea solution without surface treatment, Group III and IV immersed in tea solution with surface treatment with topical fluoride and CPP - ACP respectively which were then analysed using a spectrophotometer and they concluded that surface treatment with CPP - ACP and topical fluoride significantly reduced the stain absorption.

**Robert L.Karlinsey et al** in **2010**[42] conducted an in vitro remineralization/ demineralization study evaluating the reversal of 'white spot' lesions in bovine enamel treated with a placebo paste (Tom's of Maine), Prevident Booster 5000, or Clinpro 5000. On the basis of the results obtained in the study, they concluded that Clinpro 5000 which contains a fluoride compatible functionalized calcium phosphate ingredient imparts superior remineralization at both the enamel surface and within the subsurface lesion relative to Prevident Booster 5000. These results suggest that the synergistic combination of fluoride plus f-TCP may provide superior dental health benefits over a dentifrice

system designed to promote faster dispersion and therefore fluoride uptake, into enamel white-spot lesions

An in vitro study was conducted by **Kelio Garcia Silva, Densie Pedrini, Alberto Carlos, Bottazzo Delbem, lilian Ferreire & Mark Cannon** in **2010**[43] to evaluate the remineralizing potential of pit and fissure sealants containing Amorphous Calcium Phosphate (ACP) and/or fluoride in artificially induced carious lesions on smooth enamel surfaces. Ten volunteers who wore acrylic palatal devices were enrolled in this 5-days double - blind study and assigned to one of the following five groups: (I) demineralized enamel slab + Fluroshield (sealant with fluoride); (II) demineralized enamel slab + Aegis (sealant with ACP); (III) demineralized enamel slab + experimental sealant with fluoride (ESF); (IV) demineralized enamel slab + experimental sealant with fluoride/ACP (ACP - F); and (V) demineralized enamel slab (control). The concentrations of fluoride, calcium and phosphorus in enamel were determined and they concluded that the pit and fissure sealants containing ACP were able to promote remineralization of artificially induced carious lesions on smooth enamel surfaces.

In **2010, Lata S, N.O Varghese, Jolly Mary Varughese**[44] conducted an in vitro study to compare the remineralization potential of fluoride and Amorphous Calcium Phosphate - Casein Phosphopeptide on enamel lesions. In this study 15 intact carious free human premolars were selected and the coronal part of each tooth was sectioned into four parts

to make 4 enamel blocks. The baseline (SMH) surface micro hardness was measured using VHN testing machine. The SMH of the demineralized samples was also evaluated after immersing the specimens in demineralized solution for 3 consecutive days. Then the 4 enamel sections of each tooth were subjected to surface treatments with fluoride varnish, CPP - ACP cream and Fluoride + CPP - ACP and a control group where no surface treatment had been done. A pH cycling was carried out which includes alternative demineralization and remineralization with artificial saliva for 5 consecutive days. After that again the SMH was evaluated to assess the remineralization potential of each surface agents and they concluded that CPP - ACP cream is effective, but to a lesser extent than fluoride in remineralizing early enamel lesions at surface level. Combination of fluoride and CPP - ACP does not provide any additive remineralization potential compared to fluoride alone. Fluoride, CPP - ACP and their combination are not effective in remineralizing the early caries at the subsurface level.

An in vivo study was conducted by **Gianmaria F. Ferrazzano, Ivana Amato, Tiziana Cantile, Giancarla Sangianantoni and Aniello Ingenito** in **2011**[45] to determine the remineralizing effect of GC Tooth Mousse on early dental enamel lesions. Study protocol consisted of 40 volunteers aged between 10 - 16 years were recruited and divided in two groups of 20 (Group A and B). In group A subjects, two demineralized enamel specimens were placed on the buccal surface of first molars and subjects were instructed to apply GC Tooth mousse only on the right sided specimen and a placebo mousse on the left, for

1 month. In Group B specimens two enamel specimens were similarly placed into the mouth and used as controls. SEM analysis revealed a diffuse and homogeneous mineral coating, reducing the surface alterations only in the demineralized specimens treated with synthetic CPPs into the mouth and they concluded that CPPs are able to promote remineralization of early enamel lesions.

**E Gjorgievska, JW Nicholson** in **2011**[46] conducted a study to determine the effects of bleaching with 16% carbamide peroxide on the structure of the enamel layer of teeth and the potential of the commercial bioactive glass Novamin in two different toothpastes (Mirawhite TC and Nanosensitive HCA) to remineralize demineralized regions of enamel. Here they considered three aspects: the extent and nature of the alterations in the enamel after application of the bleaching agent; the extent of remineralization after application of two commercial toothpastes containing bioactive glass; and whether or not there were differences between the toothpastes in terms of their effectiveness in promoting remineralization. The results in their study showed that application of 16% carbamide peroxide causes distinct morphological changes to the enamel surface which vary from mild to severe. Subsequent treatment with toothpastes containing bioactive glass Novamin resulted in the formation of a protective layer on the enamel surface, consisting of bioactive glass deposits, with only slight differences between the two brands and also causes an increase in the Ca and P content of the enamel layer.

In **2011, R.L Karlinsey[47] et al** conducted an in vitro study to evaluate the in vitro remineralization effects of four dentifrice systems using microhardness and fluoride uptake analyses. The study involved the following NaF silica-based dentifrices: 1) Placebo (0 ppm F), 2) 500 ppm F) 3, 1150 ppm F 4) 500 ppm F plus functionalized tricalcium phosphate (f-TCP) after 10 days of pH cycling , specimens were analysed for surface microhardness, enamel fluoride uptake and cross sectional microhardness. The results showed that 1150 ppm fluoride and 500 ppm fluoride plus f-TCP dentifrices significantly remineralised the enamel compared to the placebo and 500 ppm fluoride dentifrices.

In **2012, Keiko nakata, Toru nikaido, Syozi Nakashima, Nobuhito Nango, Junji Tagami[48]** conducted a study to indicate the possibility of a new approach to creating mineral density profiles, and to examine longitudinal changes in the rate of remineralization and the mineral density at 4 different depths (surface zone, lesion body, middle zone, deep zone near to sound area in enamel sub surface lesions, 8 demineralized bovine enamel dentin blocks were remineralized for 1 to 4 weeks and investigated using micro CT. Their study suggested that greater the value of the mineral density before the remineralization, the smaller the mineral density increments.

# MATERIALS AND METHODS

**MATERIALS:**

1. Normal saline (Baxter 0.9% w/v Sodium Chloride)
2. McInne's demineralizing solution consisting of l ml of 36% hydrochloric acid, 1 ml of 30% hydrogen peroxide and 0.2 ml of anesthetic ether which was freshly mixed in the ratio of 5:5:1 in a dappen dish before each application.
3. Casein Phosphopeptide - Amorphous Calcium Phosphate (GC Tooth Mousse GC$^{TM}$ Japan).
4. Tricalcium phosphate containing 0.2% w/w sodium fluoride (Clinpro$^{TM}$ Tooth crème 3M U.S.A)
5. Calcium sodium phosphosilicate (Novamin) containing bioactive glass (SHY-NM$^{TM}$ Group Pharmaceuticals India )
6. Artificial saliva ($Na_3PO_4$ 3.90mM, $NaCl_2$ 4.29mM, KCl 17.98mM, $CaCl_2$ 1.10mM, $MgCl_2$ 0.08mM, $H_2SO_4$ 0.50mM, $NaHCO_3$ 3.27mM. Distilled water is also added and the pH was set at 7. 2.)
7. Cold cure acrylic (Orthoplast India)
8. Diamond disc (Axis dental, Texas) and low speed straight hand piece and micromotor (NSK Japan)
9. Silicon carbide paper of 220, 400, 800, 1200 grit (Palm abrasives, India)
10. Nail Varnish (Lakme India)

## METHODOLOGY

### SAMPLE SELECTION

45 freshly extracted single rooted permanent mandibular premolar human teeth which were extracted for orthodontic treatments with written consent from patients, were taken for the study. The teeth selected for this study were free from dental caries, restorations or developmental defects. The samples were cleaned of calculus and soft tissues and stored in normal saline. Occupational Safety and Health Administration (OSHA) and the Centre for Disease Control and prevention (CDC) recommendations and guidelines were followed during collection, storage, sterilization and handling of extracted teeth

### SAMPLE GROUPING AND PREPARATION

| GROUP NO: | GROUP NAME | SAMPLE SIZE | SAMPLE NUMBERING |
|---|---|---|---|
| GROUP – 1 | GC TOOTH MOUSSE™ | 15 | $A_1$ $B_1$ $C_1$ $D_1$ $E_1$ $F_1$ $G_1$ $H_1$ $I_1$ $J_1$ $K_1$ $L_1$ $M_1$ $N_1$ $O_1$ |
| GROUP – II | CLINPRO TOOTH CRÈME | 15 | $A_2$ $B_2$ $C_2$ $D_2$ $E_2$ $F_2$ $G_2$ $H_2$ $I_2$ $J_2$ $K_2$ $L_2$ $M_2$ $N_2$ $O_2$ |
| GROUP – III | SHY – NM | 15 | $A_3$ $B_3$ $C_3$ $D_3$ $E_3$ $F_3$ $G_3$ $H_3$ $I_3$ $J_3$ $K_3$ $L_3$ $M_3$ $N_3$ $O_3$ |

The teeth were sectioned horizontally using a diamond disc (Axis dental, Texas) with a slow speed straight hand piece (NSK Japan) at 15,000 rpm at the level of CEJ, separating the crown part of the tooth. The cusp and occlusal surface of the crown were then removed following the same technique. Next, the mesial, distal and lingual sides of the tooth block were cut to obtain flat surfaces. Finally, the buccal side was flattened and polished using 200, 400, 800, 1000, 1200 grit abrasive paper to obtain cuboidal tooth blocks of 4mm x 4mm x 6mm from each tooth[49]. The prepared and polished buccal surface has two advantages (1) enamel surface exposed was never subjected to the natural demineralization-remineralization process in the oral cavity, (2) a flat polished surface was essential for getting accurate reading of the diagonal lengths of the indentation[50]. All other tooth surfaces except the buccal surface was painted with acid resistant nail varnish and mounted in self cure acrylic resin with buccal surface facing upward and exposed. The specimens were then stored in artificial saliva.

The samples were then stabilized individually on the micro-CT scanning machine and then scanned to determine the mineral content of enamel specimens at baseline. Similarly the samples were then placed on to the table of Vicker's Hardness Testing machine, stabilized and then indented with the indenter to determine the VHN value at baseline. After determining the baseline micro CT and micro hardness values, the specimens are stored in artificial saliva.

**Demineralization of samples:**

The specimens were then placed in McInnes demineralizing solution for the purpose of demineralization. The solution was freshly prepared in a dappen dish before use[51].

1. **First cycle of demineralization:** The demineralizing agent thus prepared was applied to the entire 45 enamel surface using a cotton applicator for five minutes. It was then washed under running tap water, dried with absorbent paper and then stored in artificial saliva for 24 hours to prevent dehydration[51].

2. **Second cycle of demineralization:** Again after 24 hours the second application of demineralizing agent was carried out again for five more minutes as described earlier for all the 45 enamel specimens and the µCT and Microhardness values were recorded to determine the mineral content and surface microhardness after second cycle of demineralization[51].

**Remineralization of samples:**

1. **First cycle of remineralization:** After demineralization all the 45 specimens were subjected to remineralization treatment with Group I, Group II and Group III remineralization pastes respectively which was applied with cotton applicator tips on the demineralized samples three

minutes twice daily for fifteen consecutive days. The samples were then washed under running tap water, stored in artificial saliva for fifteen days and then subjected to µCT and microhardness test for determining the mineral content and surface microhardness respectively after the first remineralization cycle[51].

2. **Second cycle of remineralization:** Following first cycle of remineralization, remineralization pastes were applied for fifteen more days, stored in artificial saliva and at the end of thirty days the samples were again subjected for µCT and microhardness testing to determine the mineral content and surface microhardness after second remineralization cycle[51].

The micro CT and microhardness values at the baseline, demineralization and the remineralization were then compared and statistically analyzed to evaluate the net gain or loss in minerals as well as the surface microhardness of the enamel surface.

**Procedure for micro CT testing**

µCT40 is a commercial cone beam micro CT machine manufactured by SCANCO™ Medical AG, Switzerland which can be used for dental researches[52,53]. A specimen mounted on a rotating stage is positioned between an x-ray source and the detector. The source to object distance (SOD) and source to detector distance (SDD) are selected to provide the appropriate amount of

geometric magnification. Typically, the SDD is 20 cm and the SOD ranges between 7 and 18 cm. X – ray projections are acquired by a phosphor detector, coupled to a CCD camera by a Fiberoptic taper, which reduces the size of the image. During acquisition, the computer controls the X – ray tube and specimen stage, obtaining X – ray projections at hundreds of angular positions. Scanco 40 µCT scanner was used to evaluate the mineral content in this study. The individual tooth block were placed in a resin tube with a 12 mm diameter and are stabilized in position by means of thermocol which is then scanned by µCT at 70kVp. Each tooth block was numbered on one of the varnished tooth surfaces. During scanning the tooth block was placed in the tube in such a way that the tooth block is totally stabilized and the unvarnished buccal surface faces upwards. In the scanning process, two sections at fixed distances from the upper surface region of interest (ROI) were chosen to ensure that the same sections were selected for each tooth block every time using the Scanco$^{TM}$ evaluation software available in the workstation of the scanner. The whole block was then scanned in horizontal thin sections from the top to bottom at 100µm intervals and the digitalized images were captured by a computer at 1024x1024 pixels. Linear attenuation coefficient (LAC) values of the ROI were measured. Measurements were taken on three different locations in each lesions and the mean of these measurements were used as the LAC value of the lesion.

**Procedure for microhardness testing:**

Shimadzu HMV 2000 (American subsidiary of schimadzu corporation, Japan), micro hardness tester was used for the Vickers micro hardness test. The evaluations were carried out according to manufacturer's instructions. The test specimens were placed on the stage of tester, stabilized and the area to indent was selected by focusing with 40x objective lens. After this the load of 100 g was applied on the surface of specimen for 14 seconds, taking care not to indent any areas closer to the edge of the specimen. The indentation formed was viewed and measured in the computer using Shimadzu digital software. The average microhardness of the specimen was determined and noted in VHN from five indentations to avoid any operational bias. The procedure was repeated for all the specimens in three groups.

**Statistical Analysis**

Data were analyzed using computer software, Statistical Package for Social Sciences (SPSS) version 10. Data are expressed in its mean and standard deviation (SD). The mean difference and percentage difference between cycles were also found out separately for three groups. Difference in Micro CT and Microhardness within a group after any two cycles of demineralization and remineralization was compared using paired-T test. Between groups difference in the Micro CT and Microhardness after any phase of demineralization and

remineralization cycle were compared using One Way ANOVA. If the difference was found to be statistically significant after applying ANOVA test, then Post-Hoc Duncan's Multiple Range test was performed for pairwise comparison. To display a statistical difference in pairwise comparison, alphabets were used as superscripts. In a table displaying the mean difference, if the different alphabets are encountered between two groups, then the difference is said to be statistically significant whereas if the same alphabet is encountered between two groups, then the difference between those groups are not statistically significant. For the entire test, a P value of $< 0.05$ was considered to be statistically significant. This implicates that a probability of committing a type - I error is less than 5%.

Figure – 1.1 **ARMAMENTARIUM**


Figure – 1.2


Figure – 2.1

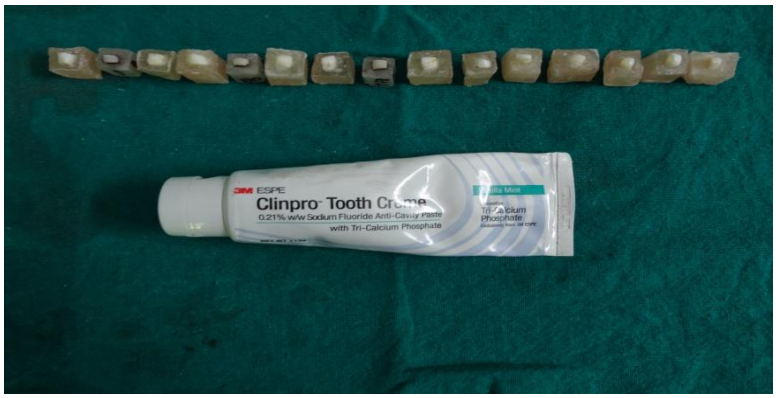



Figure -2.2


Figure -2.3

ENAMEL SPECIMENS WITH GROUP I, GROUP II, GROUP III DENTIFIRCES

Figure - 3.1: IMAGE OF MICRO CT MACHINE

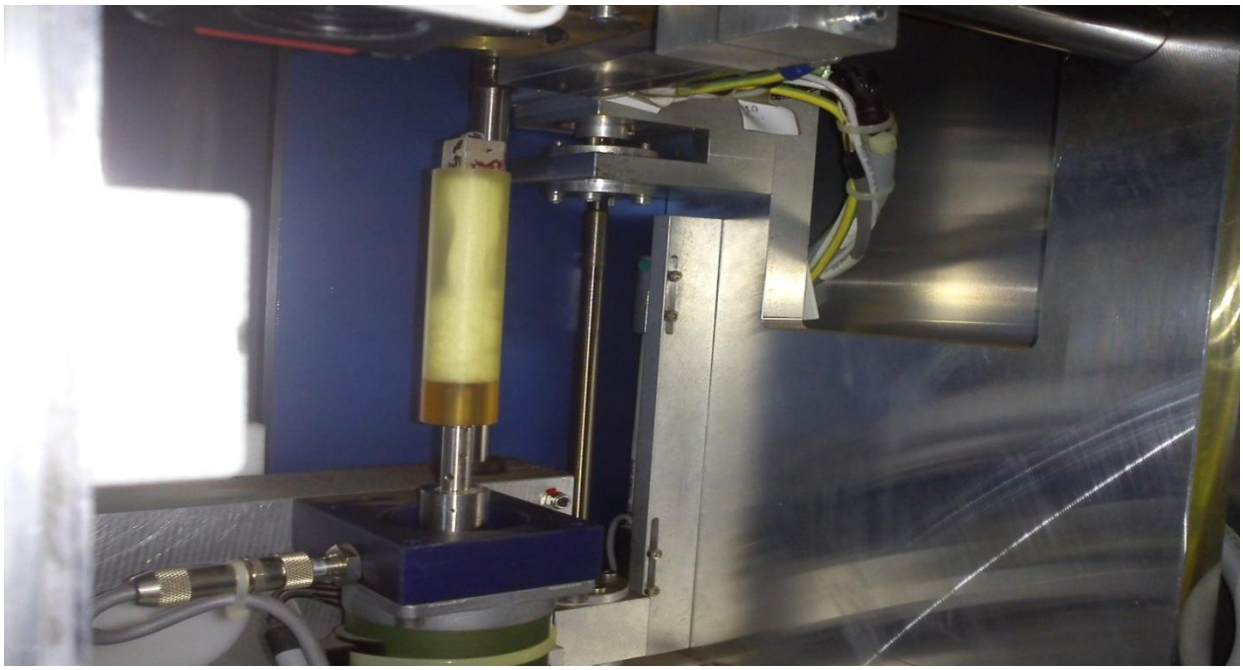


Figure – 3.2: IMAGE OF MICRO CT MACHINE WITH SPECIMEN MOUNTED

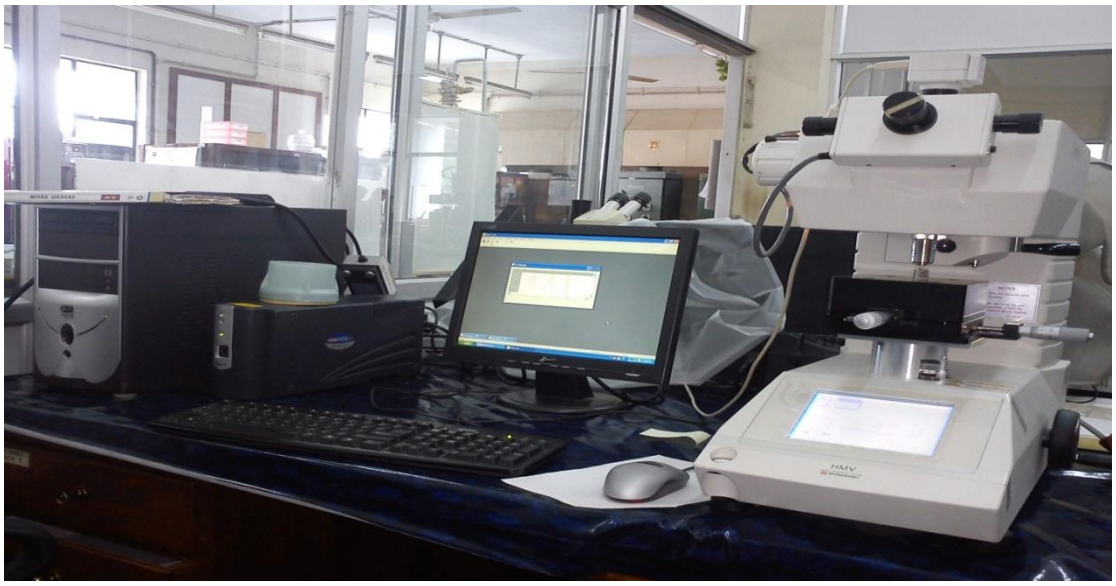


Figure – 4.1: IMAGE OF VICKER'S MICRO HARDNESS TESTING MACHINE

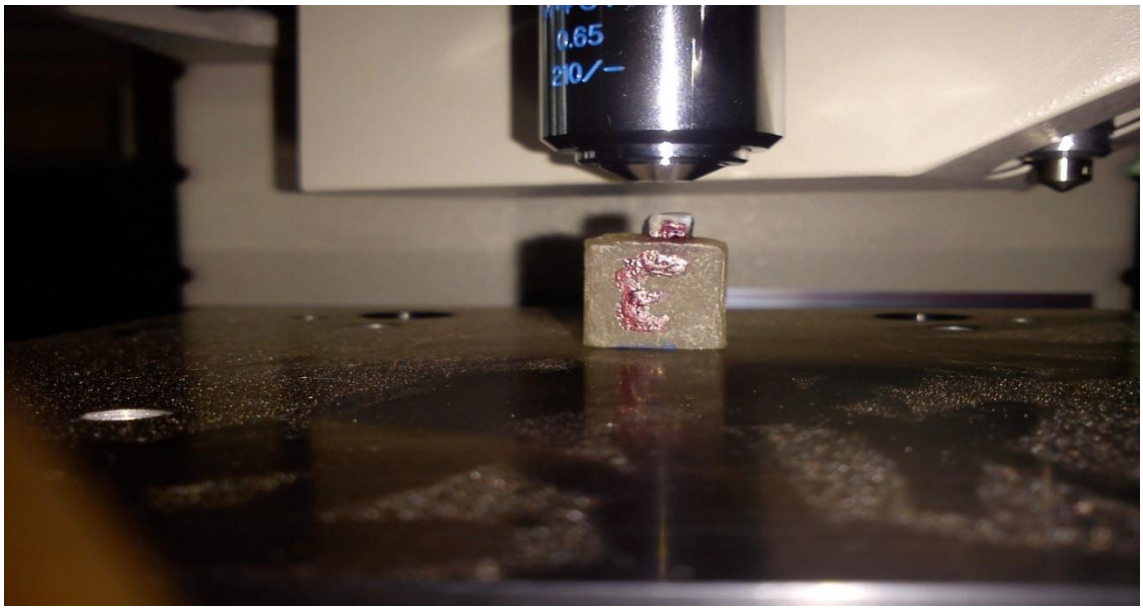


Figure – 4.2: IMAGE OF VICKER'S MICRO HARDNESS TESTING MACHINE WITH SPECIMEN MOUNTED

# RESULTS

Figure – 5.1

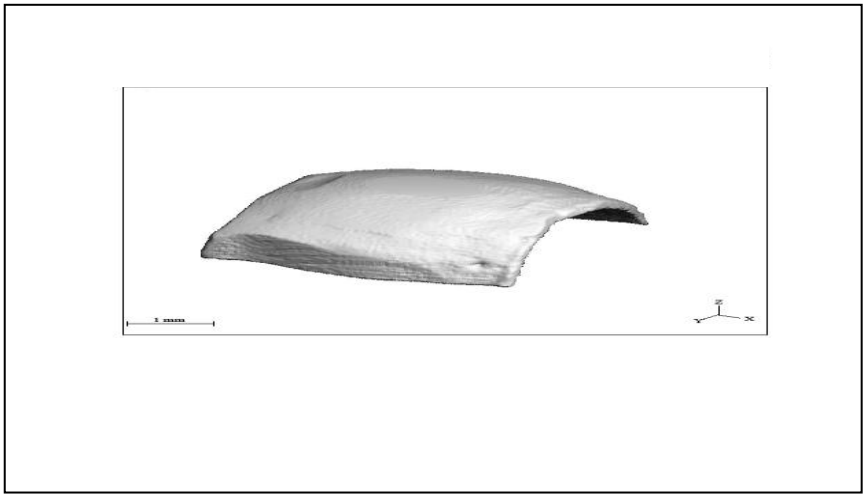


Figure – 5.2

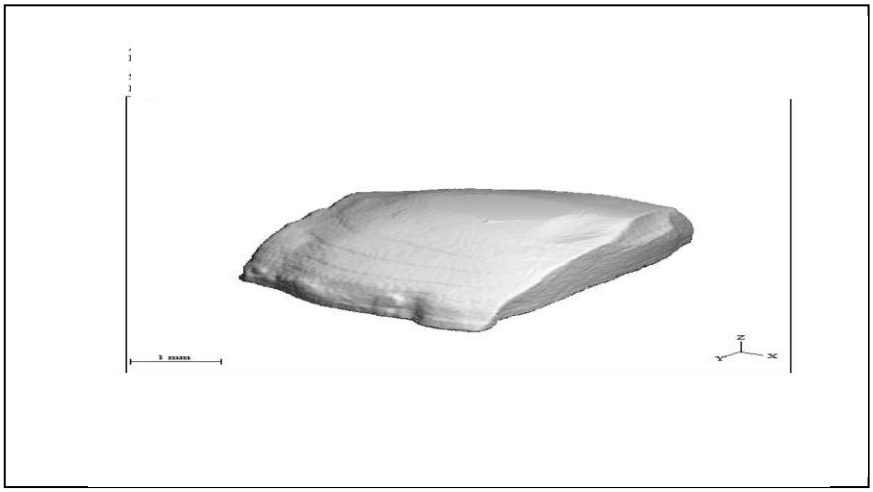


Figure – 5.3

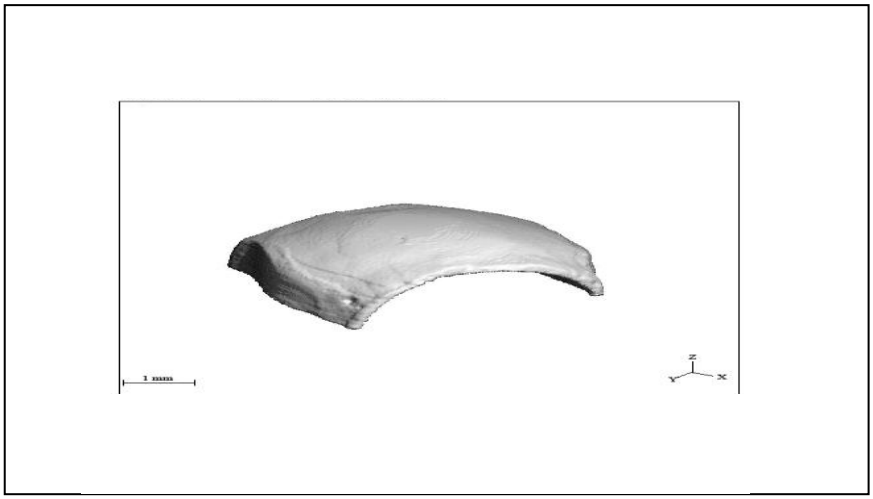




FIG 5: µ CT IMAGES OF ENAMEL SPECIMENS BY GROUP – I DENTIFRICES

Figure – 6.1

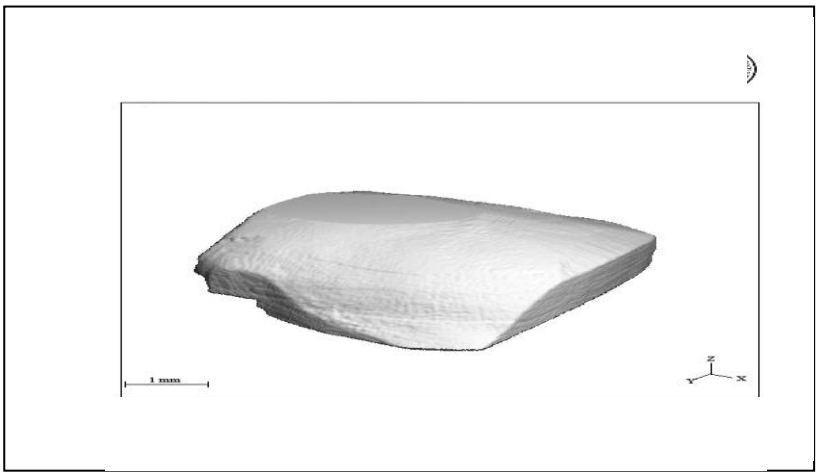


Figure – 6.2

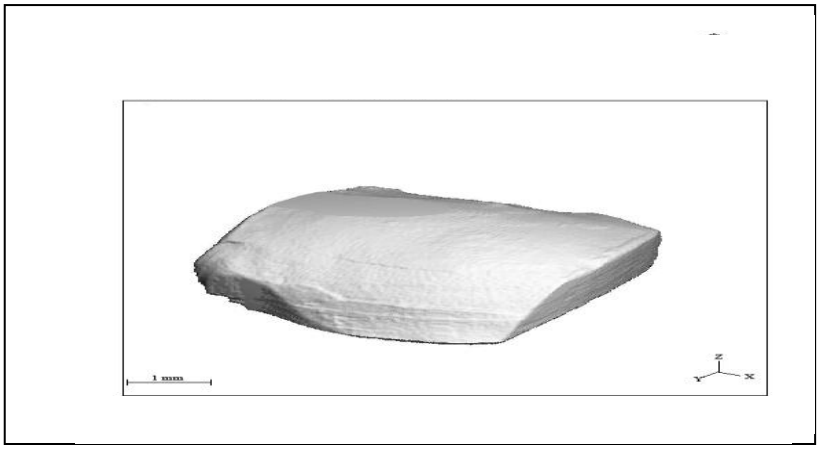


Figure - 6.3

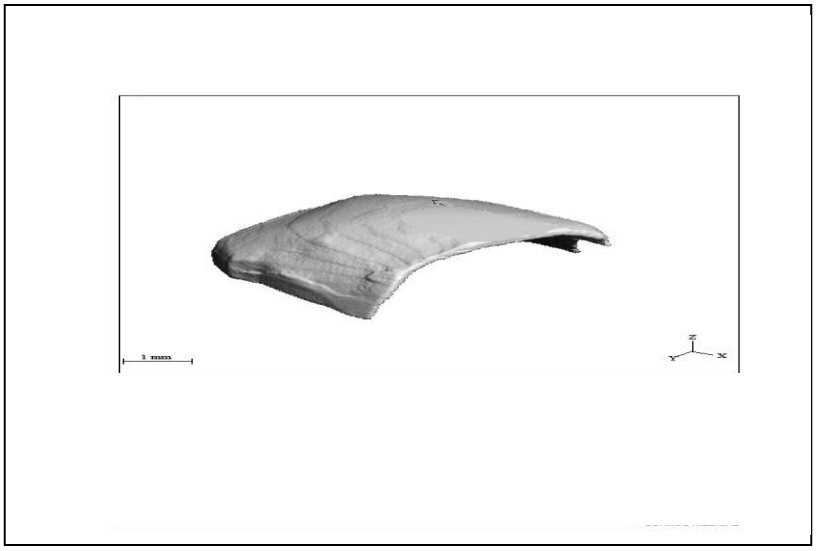




FIG 6: µ CT IMAGES OF ENAMEL SPECIMEN BY GROUP - II DENTIFRICE

Figure – 7.1

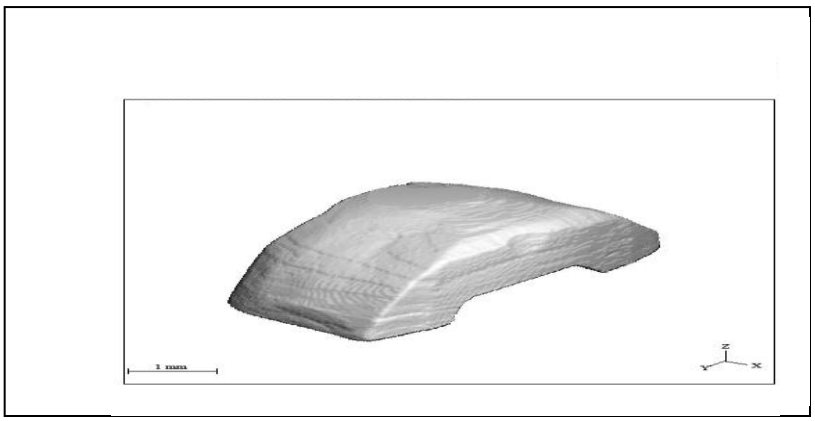


Figure – 7 .2

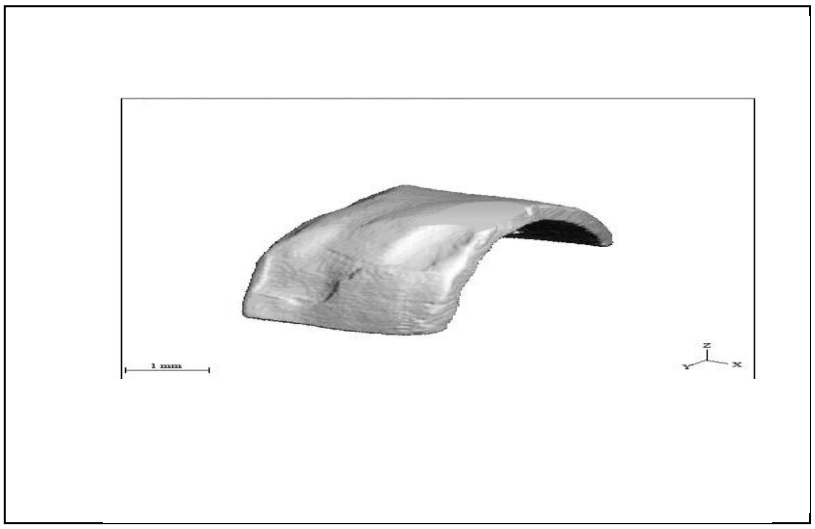


Figure – 7 .3

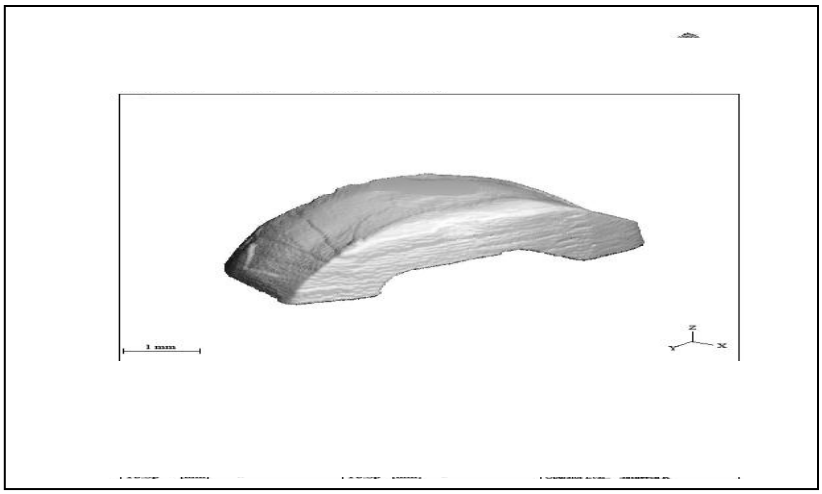




FIG 7: μ CT IMAGES OF ENAMEL SPECIMEN BY GROUP - III DENTIFRICE

Figure – 8.1

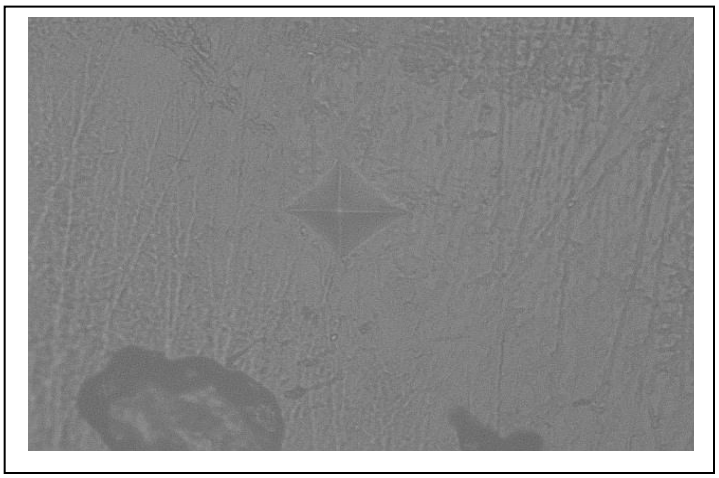


Figure – 8.2

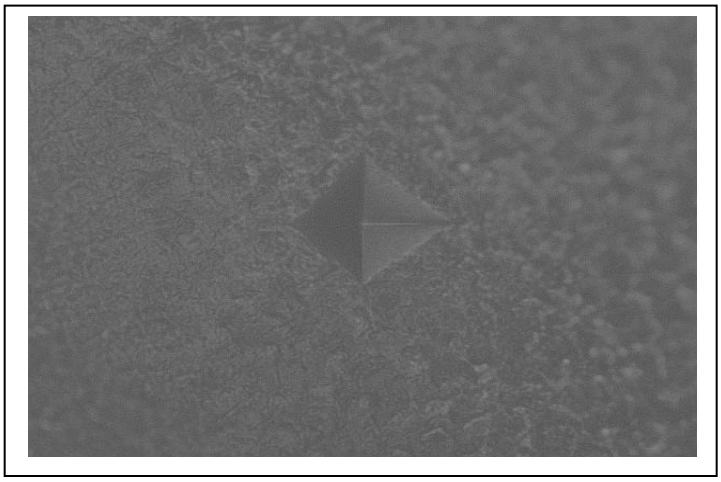


Figure – 8.3

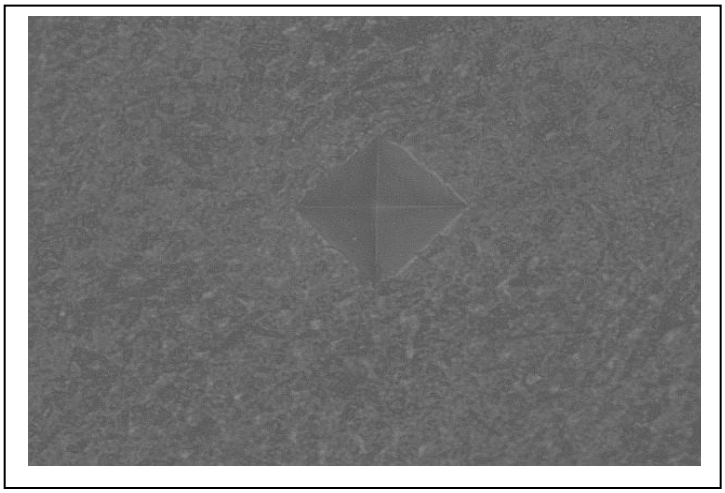




FIG 8: MICROHARDNESS INDENTATION IMAGES ON ENAMEL SPECIMEN BY GROUP - I DENTIFRICE

Figure – 9.1

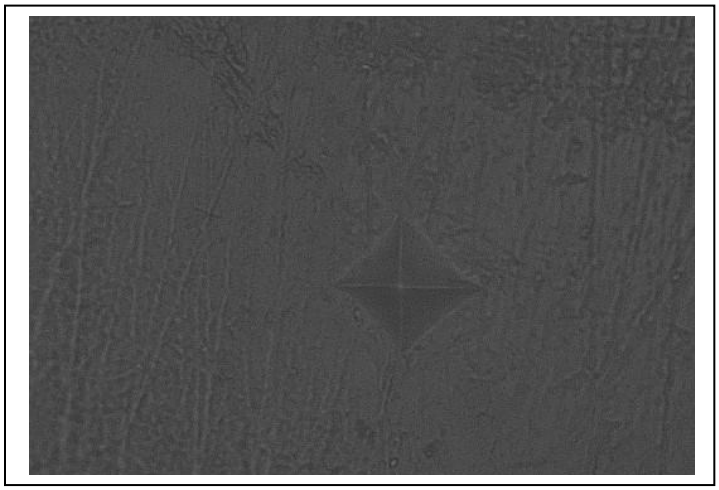


Figure – 9.2

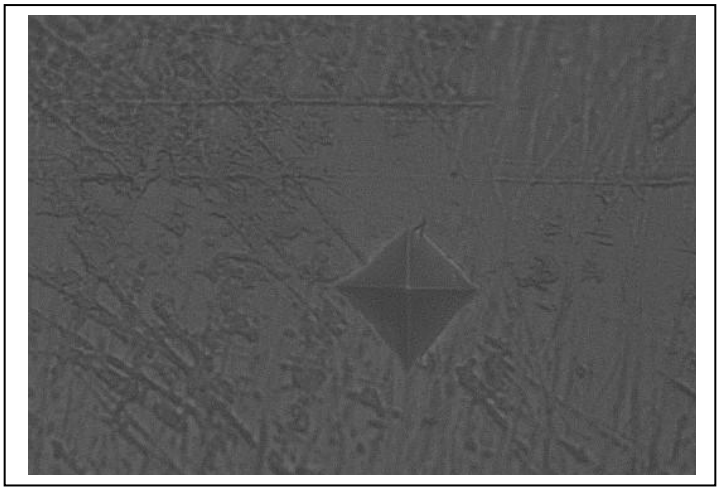


Figure – 9.3

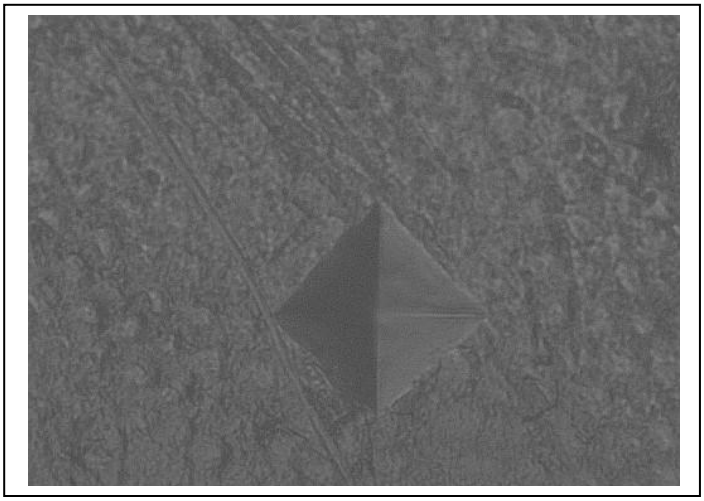




FIG 9: MICRO HARDNESS INDENTATIONS ON ENAMEL SPECIMEN BY GROUP - II DENTIFRICE

Figure – 10 .1

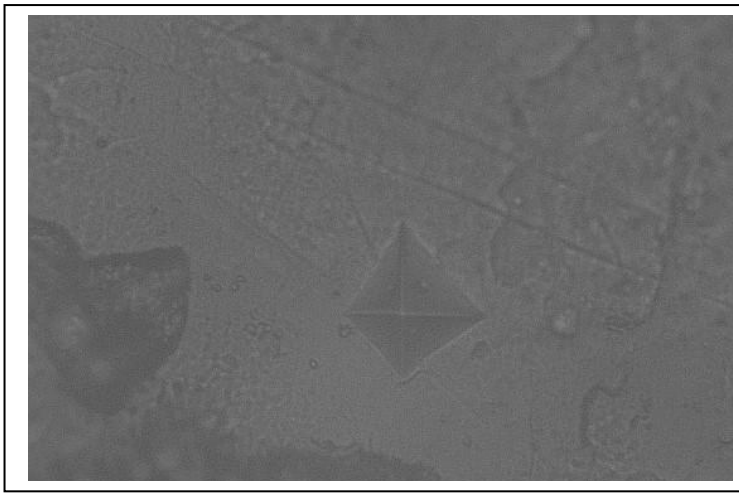


Figure – 10 .2

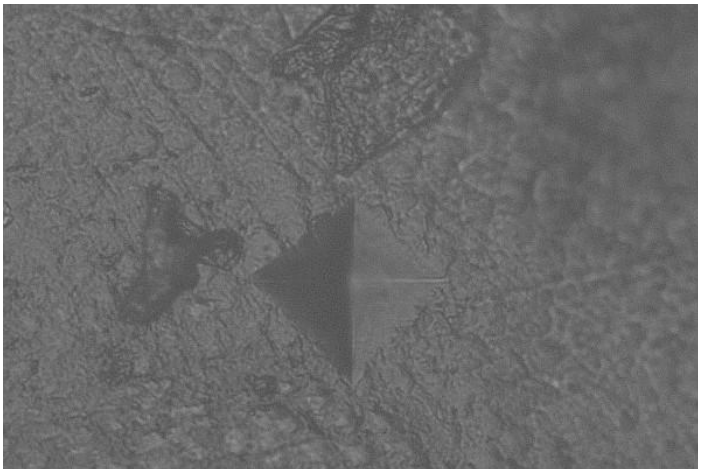


Figure – 10.3

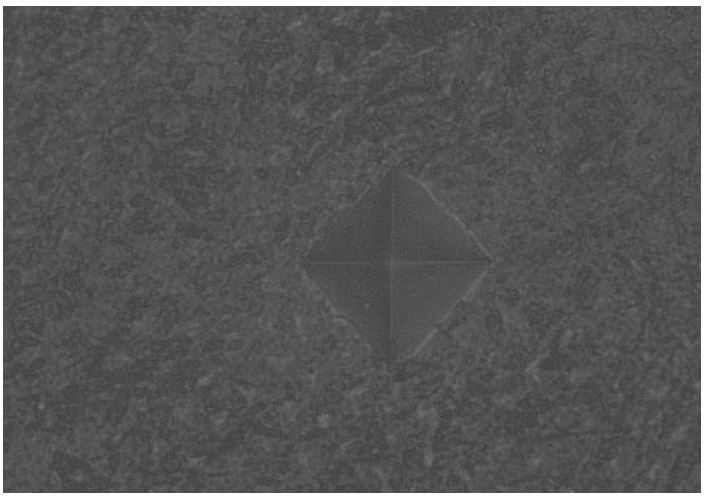


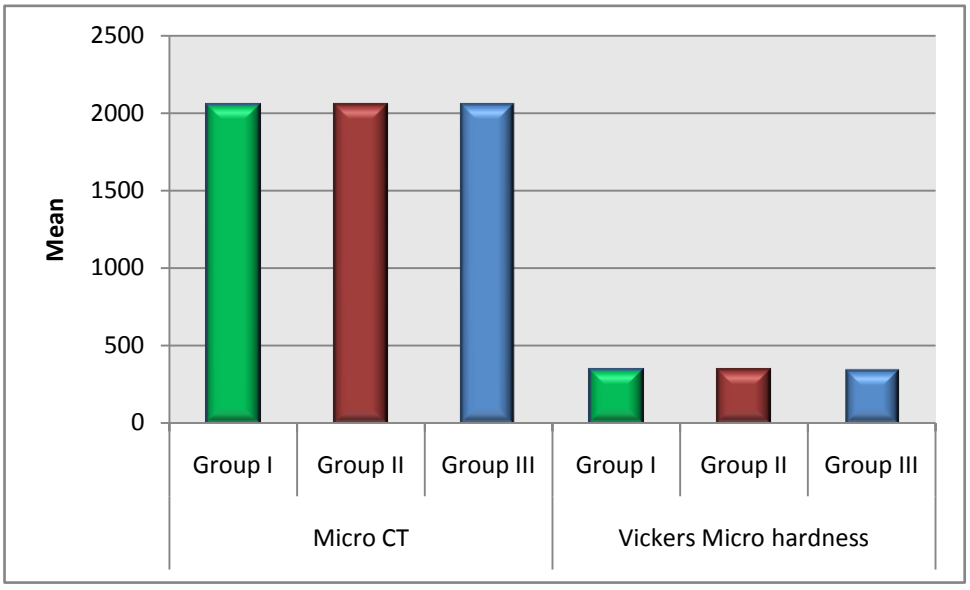
Table 1. Mean l



FIG 10: MICRO HARDNESS INDENTATION IMAGES ON ENAMEL SPECIMEN BY GROUP - III DENTIFRICE

| Parameter | Group | Mean | ± SD | F value | P value |
|---|---|---|---|---|---|
| Micro CT | Group I | 2051.10[a] | 1.76 | 0.528 | > 0.05 |
| | Group II | 2050.50[a] | 2.92 | | |
| | Group III | 2048.96[a] | 1..67 | | |
| Vickers Micro hardness | Group I | 336.36[a] | 1.39 | 0.171 | > 0.05 |
| | Group II | 333.59[a] | 1.29 | | |
| | Group III | 335.21[a] | 2.01 | | |

a – Means with same superscript within each parameter do not differ each other (Duncan's Multiple Range Test)

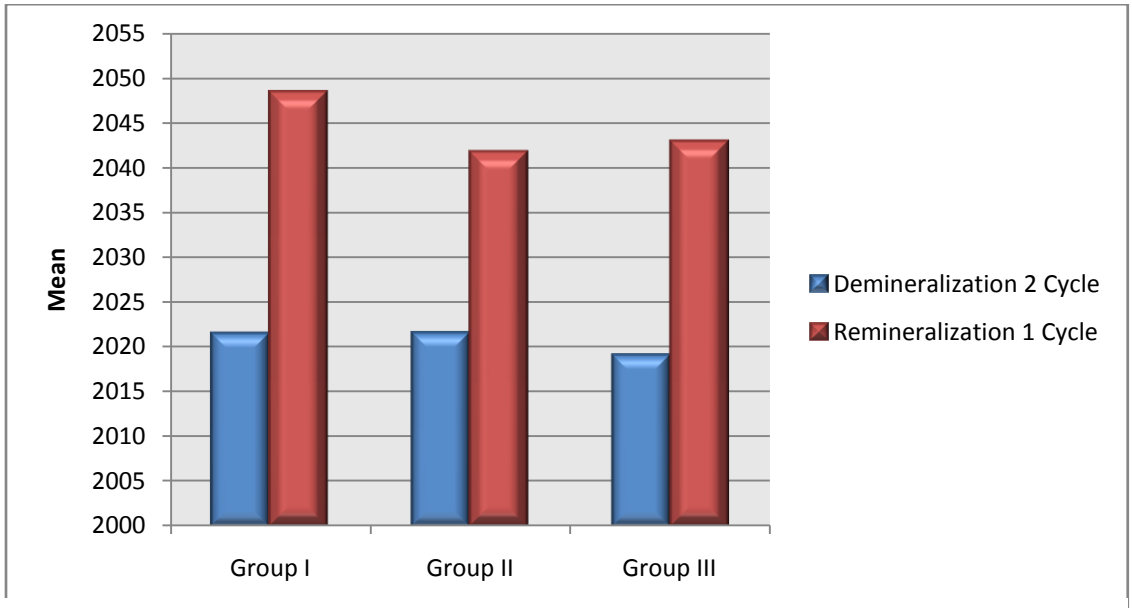



Graph No: 1 showing mean baseline values for micro CT and VHN in 3 groups

Table 2. Comparison of micro CT ($mg/cm^3$) between demineralization 2 cycle and remineralization 1 cycle in three groups

| Group | Cycle | Mean | ± SD | Mean Difference | % Difference | t value | P value |
|---|---|---|---|---|---|---|---|
| Group I | Demineralization 2 Cycle | 2021.40 | 12.09 | 36.01[c] | 1.79 | - 2.905 | < 0.05 |
| | Remineralization 1 Cycle | 2048.40 | 49.63 | | | | |
| Group II | Demineralization 2 Cycle | 2021.50 | 13.02 | 27.21[b] | 1.35 | - 2.144 | < 0.05 |
| | Remineralization 1 Cycle | 2041.70 | 40.25 | | | | |
| Group III | Demineralization 2 Cycle | 2019.00 | 14.23 | 20.91[a] | 1.03 | - 2.763 | < 0.05 |
| | Remineralization 1 Cycle | 2042.90 | 36.01 | | | | |
| ANOVA of mean difference comparing groups; F = 8.698; P < 0.01 | | | | | | | |

a, b, c – Mean difference with same superscript do not differ each other (Duncan's Multiple Range Test)

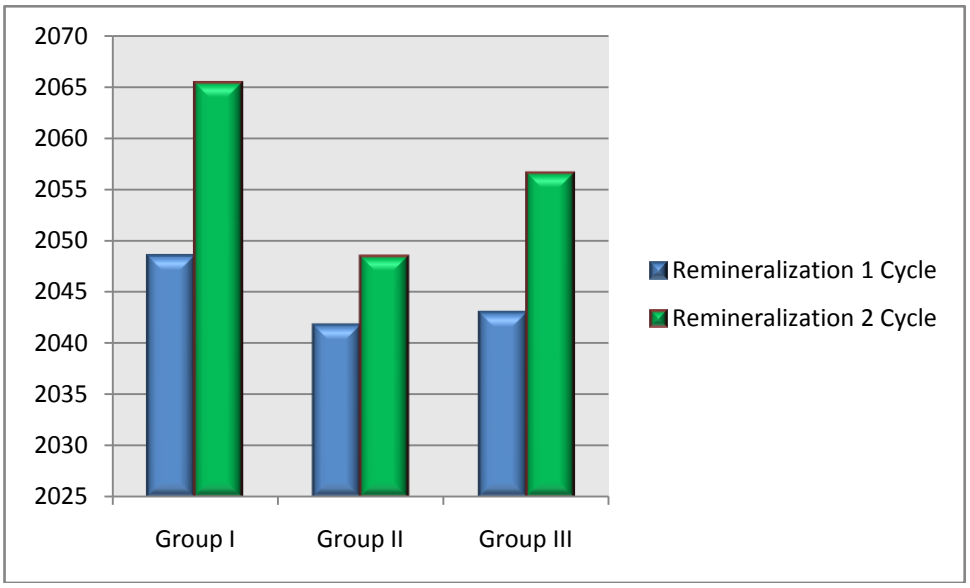



Graph No:2 showing micro CT comparison between demineralization 2 and remineralization 1

Table 3. Comparison of micro CT (mg/cm$^3$) between remineralization 1 cycle and remineralization 2 cycle in three groups

| Group | Cycle | Mean | ± SD | Mean Difference | % Difference | t value | P value |
|---|---|---|---|---|---|---|---|
| Group I | Remineralization 1 Cycle | 2048.40 | 49.63 | 16.91[c] | 0.83 | - 2.322 | < 0.05 |
| | Remineralization 2 Cycle | 2065.30 | 67.97 | | | | |
| Group II | Remineralization 1 Cycle | 2041.70 | 40.25 | 6.71[a] | 0.33 | - 2.141 | < 0.05 |
| | Remineralization 2 Cycle | 2048.40 | 49.14 | | | | |
| Group III | Remineralization 1 Cycle | 2042.90 | 36.01 | 13.61[b] | 0.66 | - 2.301 | < 0.05 |
| | Remineralization 2 Cycle | 2056.50 | 57.70 | | | | |
| ANOVA of mean difference comparing groups; F = 12.878; P < 0.01 | | | | | | | |

a, b, c – Mean difference with same superscript do not differ each other (Duncan's Multiple Range Test)

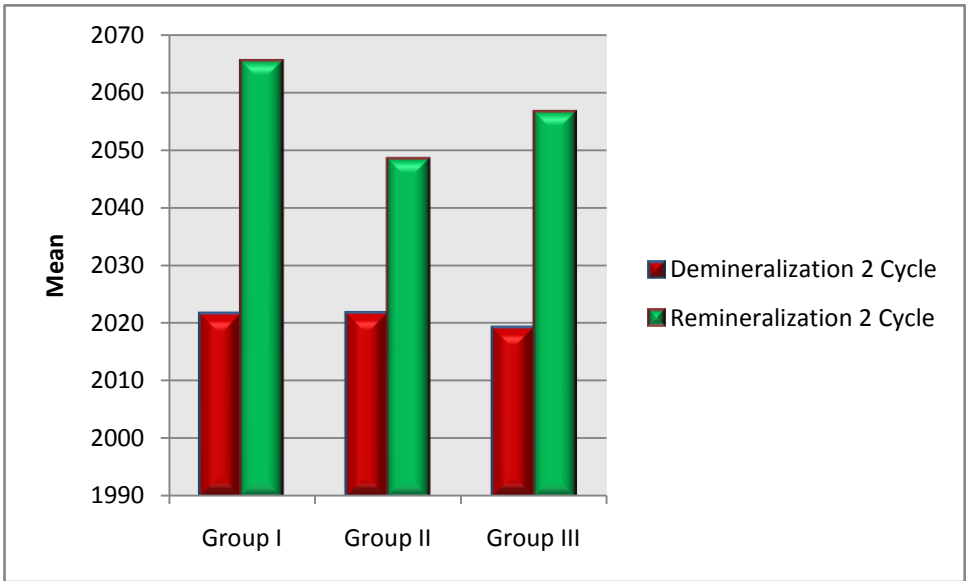



Graph No: 3 showing micro CT comparison between remineralization 1 and remineralization 2

Table 4. Comparison of micro CT ($mg/cm^3$) between demineralization 2 cycle and remineralization 2 cycle in three groups

| Group | Cycle | Mean | ± SD | Mean Difference | % Difference | t value | P value |
|---|---|---|---|---|---|---|---|
| Group I | Demineralization 2 Cycle | 2021.40 | 12.09 | 52.91[b] | 2.62 | - 2.148 | < 0.05 |
| | Remineralization 2 Cycle | 2065.30 | 67.97 | | | | |
| Group II | Demineralization 2 Cycle | 2021.50 | 13.02 | 33.91[a] | 1.68 | - 2.193 | < 0.05 |
| | Remineralization 2 Cycle | 2048.40 | 49.14 | | | | |
| Group III | Demineralization 2 Cycle | 2019.00 | 14.23 | 34.51[a] | 1.69 | 3.171 | < 0.05 |
| | Remineralization 2 Cycle | 2056.50 | 57.70 | | | | |
| ANOVA of mean difference comparing groups; F = 4.677; P < 0.05 | | | | | | | |

a, b – Mean difference with same superscript do not differ each other (Duncan's Multiple Range Test)

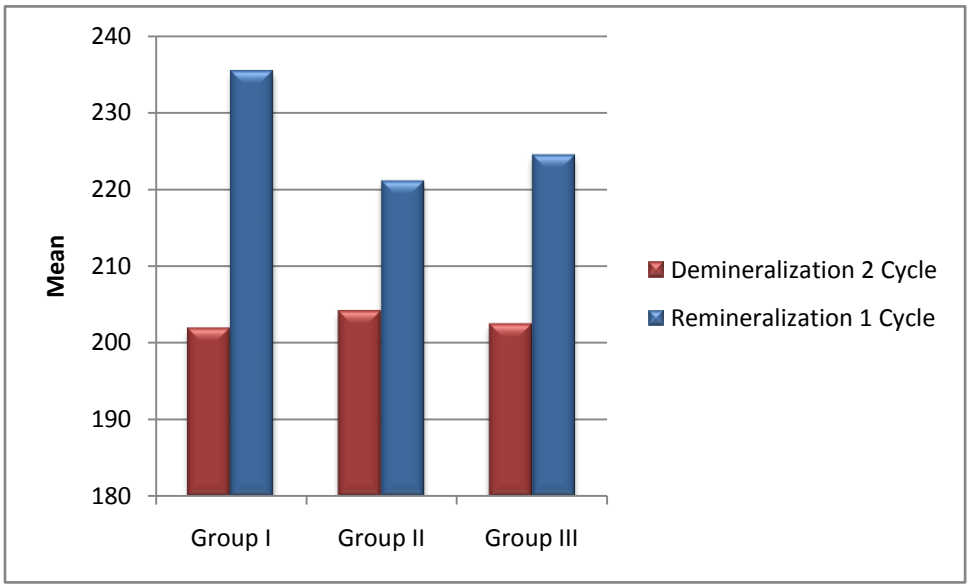



Graph No: 4 showing micro CT comparison between demineralization 2 and remineralization 2

Table 5. Comparison of Vickers micro hardness (VHN) between demineralization 2 cycle and remineralization 1 cycle in three groups

| Group | Cycle | Mean | ± SD | Mean Difference | % Difference | t value | P value |
|---|---|---|---|---|---|---|---|
| Group I | Demineralization 2 Cycle | 201.80 | 23.89 | 26.91[b] | 13.84 | - 3.847 | < 0.05 |
| | Remineralization 1 Cycle | 235.32 | 37.76 | | | | |
| Group II | Demineralization 2 Cycle | 204.06 | 23.33 | 24.41[ab] | 11.61 | - 9.257 | < 0.001 |
| | Remineralization 1 Cycle | 221.00 | 26.36 | | | | |
| Group III | Demineralization 2 Cycle | 202.37 | 22.88 | 22.02[a] | 10.88 | - 22.131 | < 0.001 |
| | Remineralization 1 Cycle | 224.38 | 22.51 | | | | |
| ANOVA of mean difference comparing groups; F = 3.998; P < 0.05 | | | | | | | |

a, b – Mean difference with same superscript do not differ each other (Duncan's Multiple Range Test)

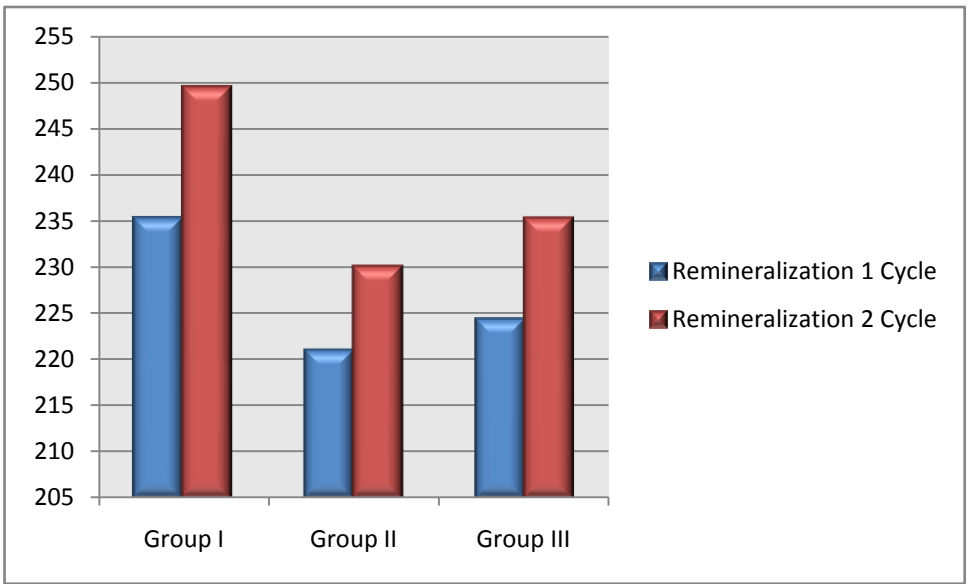



Graph No: 5 showing VHN comparison between demineralization 2 and remineralization1

Table 6. Comparison of Vickers micro hardness (VHN) between Remineralization 1 cycle and remineralization 2 cycle in three groups

| Group | Cycle | Mean | ± SD | Mean Difference | % Difference | t value | P value |
|---|---|---|---|---|---|---|---|
| Group I | Remineralization 1 Cycle | 235.32 | 37.76 | 12.11[b] | 5.47 | - 12.911 | < 0.001 |
| | Remineralization 2 Cycle | 249.42 | 36.13 | | | | |
| Group II | Remineralization 1 Cycle | 221.00 | 26.36 | 9.01[a] | 3.84 | - 7.565 | < 0.01 |
| | Remineralization 2 Cycle | 230.02 | 25.22 | | | | |
| Group III | Remineralization 1 Cycle | 224.38 | 22.51 | 11.88[b] | 5.29 | - 7.505 | < 0.001 |
| | Remineralization 2 Cycle | 235.26 | 21.69 | | | | |
| ANOVA of mean difference comparing groups; F = 4.298; P < 0.05 | | | | | | | |

a, b – Mean difference with same superscript do not differ each other (Duncan's Multiple Range Test)

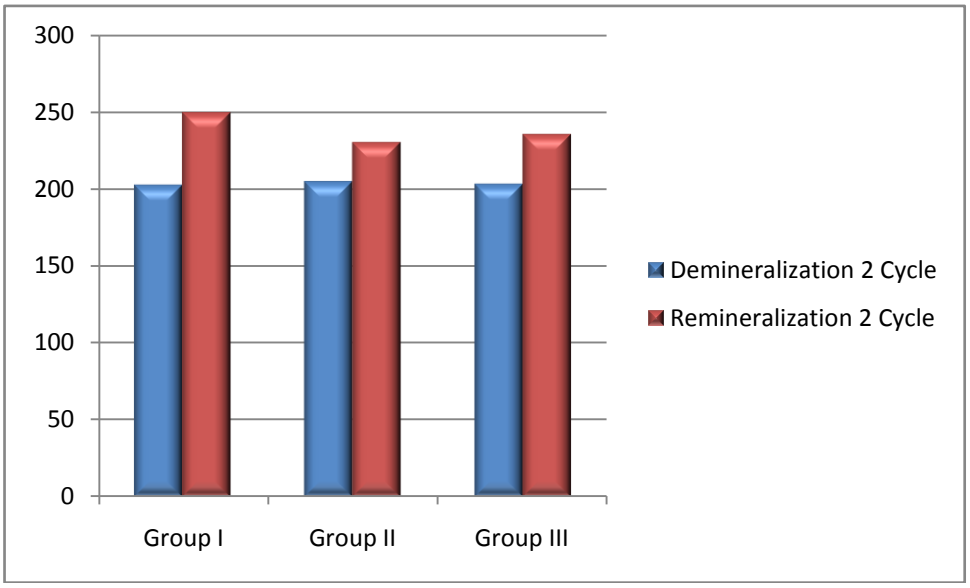



Graph No: 6 showing VHN comparison between remineralization 1 and remineralization 2

Table 7. Comparison of Vickers micro hardness (VHN) between demineralization 2 cycle and remineralization 2 cycle in three groups

| Group | Cycle | Mean | ± SD | Mean Difference | % Difference | t value | P value |
|---|---|---|---|---|---|---|---|
| Group I | Demineralization 2 Cycle | 201.80 | 23.89 | 39.02[b] | 20.07 | - 6.006 | < 0.01 |
| | Remineralization 2 Cycle | 249.42 | 36.13 | | | | |
| Group II | Demineralization 2 Cycle | 204.06 | 23.33 | 33.41[a] | 15.88 | - 17.512 | < 0.001 |
| | Remineralization 2 Cycle | 230.02 | 25.22 | | | | |
| Group III | Demineralization 2 Cycle | 202.37 | 22.88 | 33.89[a] | 16.75 | - 22.751 | < 0.001 |
| | Remineralization 2 Cycle | 235.26 | 21.69 | | | | |
| ANOVA of mean difference comparing groups; F = 4.278; P < 0.05 | | | | | | | |

a, b – Mean difference with same superscript do not differ each other (Duncan's Multiple Range Test)




Graph No: 7 showing VHN comparison between demineralization 2 and remineralization 2

# DISCUSSION

The earliest clinically detectable sign of dental caries is the incipient enamel lesion known as a white spot, which becomes detectable radiographically as the lesion progresses into the enamel and dentin[54]. White spot lesion is an area of demineralized enamel that usually develops because of prolonged plaque accumulation[55]. Over time when plaque accumulates and aciduric bacteria colonize, active white spot lesions are produced. If left untreated, a cavitated carious lesion can develop. The lesion appears opaque as a result of loss of subsurface enamel, resulting in loss of enamel translucency[56]. At this stage, before cavitation, therapeutic measures can be applied to reverse or arrest the progression of the lesion[1]. Early diagnosis enables small lesions to be identified so that remineralization of lesions by preventive measures can be attempted.

Noninvasive intervention can convert a lesion from an active to an inactive state. The clinician needs to be able to monitor the outcome of noninvasive measures and in cases where there is evidence of lesion progression, make a timely decision to intervene, using minimally invasive techniques and restoring damaged tooth structure without weakening the tooth. Applying strategies to control, arrest, or reverse the disease process can reduce the economic burden, pain, and suffering of placing and replacing restorations. This modern, conservative approach to clinical management of dental caries, which has been evolving in the recent past, has necessitated a critical appraisal of methods used today for clinical detection of carious lesions. Complementing traditional

diagnostic methods with advanced, more sensitive methods will improve caries diagnostic efficiency and hence the dental care and treatment of patients.

Methods currently in use for the clinical diagnosis of caries are patient history, visual inspection , tactile examination with a blunt probe, tooth separation, fiber optics, radiographs, nutritional analysis and salivary analysis[57].

Recent advances in caries diagnosis includes impaired radiographic imaging techniques, RVG, Digital Subtraction Radiography, Light enhancement techniques like FOTI, DIFOTI, Light scattering, Ultraviolet illumination, Laser fluorescence, Quantitative laser fluorescence, Autofluorescence, other techniques like electrical conductance measurement, Ultrasonic imaging, Optical coherence tomography, ultrasonics etc[57]. Recent advances in the molecular studies revealing the mineral loss and gain during caries process have provided a pathway for the success of the new technology.

Demineralization is basically the loss of mineral apatite from the enamel[58,59]. When organic acids, such as lactic and formic acids, are produced by acidogenic bacteria, they diffuse in various directions through the enamel and dentin organic matrix into underlying tissues[60,61]. The organic matrix accelerates the demineralization process by providing permeable channel networks for acid invasion[59]. When the acid reaches a susceptible site on the crystal surface, minerals dissolve into the surrounding aqueous

phase. This is the first step of demineralization, which takes place at the atomic level before any clinically visible sign is observed. Critical pH, which is about 5.5 for enamel, is the pH level at which demineralization occurs. If the calcium and phosphate supersaturation levels are restored and aided by fluoride, minerals will diffuse into the tooth and deposit a new, more acid-resistant layer on the crystal remnants in the non-cavitated lesion[62].

Since the 1940s, it has been noted that the outermost enamel layer is the most resistant to dissolution[63]. Two mechanisms have been proposed for the formation of this hypermineralized surface layer of incipient lesions. The first is the deposition of fluoride and other ions from saliva and the other is the outward diffusion of minerals and ions from the subsurface lesion that would be deposited in the surface layer[63,64].

Over time, researchers have been more interested in utilizing various remineralization techniques, and in detecting incipient lesions as early as possible to prevent further cavitations[65]. Remineralization is the body's natural repair process of enamel rod structure following acidogenic episodes[66]. The basic mechanism of remineralization involves the diffusion of calcium and phosphate from saliva and other topical sources aided by fluoride to build a hypermineralized, acid-resistant, fluoroapatite - like layer on the existing crystal remnants, which act as remineralization nuclei. This is one crucial mechanism of action of fluoride in the inhibition and reversal

of the caries process[67]. Enamel surface is in a dynamic equilibrium with its local, oral environment with a constant movement of ions. Except under unusual circumstances, demineralizing conditions in the mouth are transient. The extent of demineralization relates inversely with the duration of the exposure and frequency of acid attacks. An increased amount of saliva minimizes the effects of acids produced by bacteria. This is attributed to the saliva that washes out cariogenic residues and exhibits a buffering capacity[68].

Remineralization is studied from two perspectives. The first is the process of filling the enamel defects formed due to demineralizing, acidogenic episodes by mineral deposition. In this case, the demineralization and remineralization magnitude determines whether a lesion would develop or the tooth remains sound. The second is repairing an incipient lesion that has already developed but still could be filled completely or partially with calcium phosphates under suitable remineralizing conditions[69].

The aim of this study was a comparative evaluation of the remineralizing potential of three different commercially available dentifrices on demineralized tooth surfaces evaluated using micro-CT and microhardness testing.

The buccal side of the sample was flattened and polished using 200, 400, 800, 1000, 1200 grit abrasive paper to obtain a comparatively flat surface to avoid any

operational bias during Vicker's Micro Hardness measurement and it was in accordance with the study done by H E Darshan and Baljeet[35,51].

Demineralization with McInne's bleaching solution was done in two cycles as it has been proved in the previous studies that 24 hours later, only after the completion of the second cycle of demineralization, there was a significant reduction in VHN and it has also been reported that greater frequency of application of McInne's bleaching agent could create lower values of VHN[35,70]. One recent review by Attin et al[70] showed that a significant higher number of bleaching treatments resulted in enamel microhardness reduction when artificial instead of human saliva was used for the storage of enamel specimens in the intervals between the bleaching applications.

Various techniques have been followed to neutralize the effect of bleaching; the use of baking soda, prophylactic paste containing fluoride, APF gel and use of copious amount of water. In the present study water has been used for this purpose[51].

Artificial saliva was used for storing the specimens in between the bleaching cycles, because it is believed that artificial saliva contributed to a slight increase in the microhardness, after demineralisation[35].

The remineralization treatment regimen of 3 minutes twice daily application was employed as per the manufacturer's recommendations and it has also been proved in the

previous studies that the longer the duration of the remineralizing agent in contact with the teeth, the better was the remineralization[35,51].

The three different remineralizing agents used in this study were Casein phosphopeptide - Amorphous calcium phosphate (GC Tooth Mousse), 0.21% sodium fluoride - Tricalcium phosphate (Clinpro tooth crème) and Calcium Sodium Phosphosilicate (Novamin) containing dentifrice (SHY -NM).

Microcomputed tomography (μ-CT) is a microscopic version of computed tomography that allows non - destructive visualization of the morphological characteristics of teeth and the determination of the mineral content in teeth and bones. In dental materials research, μ-CT is used to provide both qualitative and quantitative data. Currently, μ-CT is used for 3-D rendering and quantification of mineral content in tissues in medicine and dentistry. X-ray microtomography studies of biological hard tissues can be divided into studies of cortical and cancellous bones and those of teeth. Teeth and cortical bone studies concentrate on the measurement of the degree of mineralization, while cancellous bone studies concentrate on trabecular, morphological measurements. Mineral concentrations in cortical bones can be obtained by converting their linear attenuation coefficients while assuming they are comprised of protein and pure hydroxyapatite, and by using calculated, mass-attenuation coefficients. Apart from that the most valuable advantage of micro-CT is the measurement of whole tooth,

permitting longitudinal experiments to be conducted and the possibility of three - dimensional non - destructive evaluations. Because of its advantages over the conventional equipments, micro CT has been used as one of the evaluation criteria in this study.

Enamel demineralization and remineralization is a surface phenomenon and occurs at surface and subsurface levels. The surface microhardness measurement therefore gives us information about surface integrity, caries progression and also helps in evaluating demineralization-remineralization process. Microhardness test was selected mainly because it was economical compared to other tests and was easily available[58]. Microhardness measurement of tooth material can be done in three different ways; Knoop's Hardness Number (KHN), Vicker's Hardness Number (VHN), and Brinnel's Hardness Number (BHN). In the present study Vicker's Hardness Number was chosen over Knoop's Hardness Number because a square shape of indent obtained in VHN was easy and accurate to measure. Even the minute changes in the square shape indent after the test could be easily detected, whereas the Knoop Hardness test gave a rhomboid shape indentation with opposing surfaces parallel to each other and difficulty in detecting the error.

One of the factors that affected the hardness measurement was the specimen's preparation, because any tilt or irregular surface would yield too large an indentation and

thus a smaller Vickers's hardness measurement. Hence five indentations were made to avoid any operational bias and then the averages of these indentations were taken for statistical analysis.

Clinicians often consider Fluoride as the ultimate tool in caries prevention. Fluoride is in no doubt one of the most important agent in promoting remineralization because low level of Fluoride in saliva and plaque help prevent and reverse caries by inhibiting demineralization and enhancing remineralization.

Marinho et al stated that topical application of highly concentrated fluoride formulations has been found to be effective in caries prevention. These treatments produced a distinct calcium fluoride like layer on the enamel which is dissolved over time by oral fluids and partly incorporated into the enamel[82]. But excessive usage of fluorides either in topical or systemic form for caries prevention can lead to an increased risk of fluorosis. On the other hand, high levels of surface fluoride can increase resistance to carious lesion formation and to dental erosion. The usage of routine fluoridated dentifrice containing about 1000 - 1500 ppm fluoride by children was restricted in many countries, but many studies had shown that if the fluoride concentration is less than 1000 ppm, the dentifrice will not help in remineralization. It is therefore very essential to understand the relationship between calcium, phosphate and fluoride ions in the remineralization

process. Thus the present remineralizing agents are manufactured in such a way that they provide appropriate calcium remineralization with minimal risk of fluorosis.

The concept of Casein phosphopeptide – Amorphous calcium phosphate (CPP - ACP) as a remineralizing agent was first postulated by Reynolds in 1998. CPP - ACP nanocomplexes has been proven to be efficacious in both the prevention and reversal of enamel lesions in caries models[72,73,74]. It has been shown that CPP - ACP can be used to prevent demineralization and promote remineralization of early enamel lesions and it has a short term remineralizing effect in clinical in situ trials and long term caries - preventing effect in the in vivo randomized control trial[75,76].

The CPP has been shown to not only stabilize ACP, but also to deliver and localize ACP at the tooth surface[74]. The proposed anticariogenic mechanism for CPP - ACP is by the localization of amorphous calcium phosphate on the tooth surface, which buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to the tooth enamel and thus preventing demineralization and enhancing remineralization. It has also been stated that CPP - ACP as a valid preventive system against demineralization of early enamel lesions. Due to these reasons CPP - ACP formulation from GC Tooth mousse (GC$^{TM}$) Japan has been included in this study.

In the present study, the enamel specimens were subjected to a demineralization in two cycles[36,70]. After the second cycle of demineralization, the VHN value as well as the LAC values drastically dropped within a range of 201 - 204 VHN and 2019 - 2021 mg/cm3 respectively. The VHN values obtained in this study were almost in similar range [162 - 183] when compared to a study conducted by Lata. S et al[44]. The LAC values obtained in this study are (were) in similar range [1500 - 3960] when compared to a study conducted by E.C.M Lo et al[49]. The difference in values may be due to the difference in methodology and the demineralizing agents used in this study.

In the present study when the demineralized enamel specimens were subjected to a remineralizing cycle with CPP – ACP for fifteen days (First cycle of remineralization), the VHN values showed a marginal recovery in micro hardness of 235.32 $\pm$ 37.76 VHN from 201.80 $\pm$ 23.89. When the remineralization cycle was repeated for fifteen more days, the VHN value increased to 249.42 $\pm$ 36.13 which was statistically significant ($P<0.05$). Similarly when the demineralized enamel specimens were subjected to the first cycle of remineralization, the LAC values showed a marginal recovery within a range of 2048.40 $\pm$ 49.63 mg/cm3. When the remineralization cycle was repeated for fifteen more days, the LAC value increased to a range of 2065.30 $\pm$ 67.97 mg/cm3 which was statistically significant ($P<0.05$).

Mitra Hedge et al[23] in 2007 evaluated the remineralization of enamel by CPP – ACP using Energy dispersive x-ray analysis. The authors concluded that

CPP – ACP was successful in remineralizing sub surface enamel and the extent of remineralization achieved was dose dependent and increased with increase the time of exposure and duration of the study. Thus in the present study a greater increase in the microhardness was seen since the specimens were subjected to thirty days cycle compared to the five days study done by Lata. S. Although most of the remineralizing solutions were supersaturated with respect to amorphous and crystalline calcium phosphate pastes, the solutions which were stabilized by the CPP - ACP, such as in GC Tooth Mousse, the spontaneous precipitation of calcium phosphate did not occur and thus the longer the duration of solution in contact with the teeth, the better was the remineralization. According to manufacturer's instructions for the maximum benefit the GC Tooth Mousse should be left on the tooth surface as long as possible.

Fluoride ions helps in remineralization by the formation of fluorapatite in enamel in the presence of calcium and phosphate ions produced during enamel demineralization[76]. Fluoride ions also help in the remineralization of previously demineralized enamel surface if enough calcium and phosphate ions are present in the saliva or plaque. Thus the f-TCP and 950 ppm sodium fluoride containing dentifrice have an added advantage of fluoride in addition to novel functionalized tricalcium phosphate thus aiding in enhanced remineralization.

The f-TCP contains 2% sodium lauryl sulfate which prevents calcium phosphate reaction with fluoride and formation of calcium fluoride. As a result fluoride, calcium and phosphate are available in aqueous form for remineralization process[42].

In the present study the microhardness of enamel specimens when treated with fTCP + NaF showed a mean increase of 221.00 ± 26.36 from 204.06 ± 23.33VHN after 15 days and to 230.02 ± 25.22 VHN after 30 days which was statistically significant (P <0.05). Similarly the LAC value also increased to 2041.70 ± 40.25 from 2021.50 ± 13.02 after 15 days and to 2048.40 ± 49.14 after 30 days treatment which was statistically significant as well. (P = <0.05).

In another study conducted by Karlinsey in 2011[47], they compared the effect of f-TCP + 500 ppm of fluoride combination with three other remineralizing agents and measured changes in surface microhardness of the enamel. At the end of 10 days of treatment the microhardness of enamel increased by 106.2 ± 7.4 VHN. In a study conducted by Karlinsey et al,2010[47] he compared 5000 ppm containing dentifrice with clinpro 5000( 5000ppm fluoride + f-TCP). The results showed that Clinpro 5000 showed a mean increase of 105.6 ± 5.6 VHN, in microhardness of enamel after 10 days. In the present study the micro hardness of enamel treated with f-TCP + NaF showed a mean increase of 230.02 ± 25.22 from 204.06 ± 23.33 after 30 days which was statistically significant (P<0.05). The difference in the values could be attributed to the number of

days the remineralization was carried out and also the remineralizing agents used in both of the studies.

Novamin is a bioactive glass - ceramic material, which falls into a class of newer agents that provide calcium and phosphate upon reaction. Here the active ingredient is a Calcium Sodium Phosphosilicate (CSP) that reacts when exposed to aqueous media and provides calcium and phosphate ions that forms a hydroxyl - carbonate apatite (HCA) with time. There are many studies in literature to support Novamin as a successive desensitizing agent. However only a few studies are available to support the remineralizing action of Novamin on enamel[77,78].

The micro hardness of enamel specimens when treated with CSP toothpaste showed a mean increase of 224.38 ± 22.51 VHN from 202.37 ± 22.88 after 15 days and to 235.26 ± 21.69 after 30 days treatment which was statistically significant. LAC values also increased to 2042.90 ± 36.01 from 2019.00 ± 14.23 after 15 days and to 2056.50 ± 57.50 after 30 days which was statistically significant as well.

The present study compared the change in microhardness and mineral content following treatment with CPP – ACP, f-TCP + NaF and Novamin containing dentifrice over 30 days. It was observed that after 30 days, no significant difference was seen between the CPP – ACP and Novamin groups, but mean increase in microhardness and mineral content was more in CPP – ACP group compared to Novamin group. In a study

by Rehder Neto et al and colleagues[37], they assessed whether pastes containing CPP – ACP and CSP could control artificial caries lesion progression. The results of their study showed that CSP containing paste had 7.1% increase in mineral content when compared with CPP - ACP containing paste which showed only 3.2%. The authors therefore concluded that CSP containing paste, CPP - ACP + F, were superior to CPP - ACP paste. This may be due to the difference in remineralizing agents used in both studies and the time period used in both the studies.

In 2011, Turssi CP et al[79] conducted a study to compare the remineralizing potential of CPP - ACP and CSP containing paste on acid softened enamel. They compared 4 products with control, i) CPP - ACP [MI paste, GC America]; ii) CPP – ACP + Fluoride [MI Paste plus, GC America]; iii) CSP [Tooth revitalizing paste, Oravive]; iv) Fluoridated dentifrice [FD Sensodyne cool gel GSK]; v) Control [CO, unexposed to any product]. The results obtained showed that, the increase in SMH in CPP - ACP & CSP group did not differ significantly and was higher than control group. The present study also showed similar results, where no significant difference in microhardness values was observed between the CPP – ACP and Novamin group.

Karlinsey et al[9] compared MI Paste plus [CPP-ACP], Thermamed SOS, f-TCP + NaF. After 10 days of pH cycling, the results showed that Thermamed SOS was superior to all the agents used, however equivalent to Thermamed SOS was f-TCP + NaF

combination which was superior to CPP - ACP. In the present study remineralization potential of CPP - ACP, f-TCP + NaF, Novamin over a period of 30 days were compared. In contradiction to study mentioned above, the present study showed CPP – ACP was superior to f-TCP + NaF and Novamin group. f-TCP + NaF and Novamin group showed no significant difference in the mean values of micro CT and microhardness after treatment with the three different agents.

All the samples used in the study showed a significant reduction in LAC and VHN values after the first cycle of demineralization which was not in accordance with the previous studies and this may be due to alteration in the pH value[80]. When the demineralizing cycle was repeated after 24 hours (the second cycle of bleaching) there was significant reduction in the LAC as well as VHN, which was significant.

The values obtained after the application of remineralizing agent for fifteen days (first cycle of remineralization) showed an increase in LAC and VHN which was significant. When the remineralization cycle was repeated after next fifteen days (the second cycle of remineralization) there was significant increase in the LAC as well as VHN, which was also significant.

Paired t test was used to compare mean values between two cycles within each group. Analysis of variance (One Way ANOVA) was performed as parametric test to

compare mean difference between groups. Duncan's Multiple Range Test was also done as post-hoc analysis along with ANOVA to elucidate multiple comparisons.

The results of the present study showed that CPP – ACP has the better remineralizing potential among the three groups evaluated and it can be attributed due to the peculiar nature of CPP. CPP by stabilizing calcium phosphate in a metastable solution, facilitate high concentrations of calcium and phosphate ions, including $CaHPO_4$, which can diffuse into the enamel subsurface lesion. The CPP will also maintain the high activities of the free calcium and phosphate ions during remineralization through the reservoir of bound ACP. The bound ACP, by being in dynamic equilibrium with free calcium and phosphate ions, will maintain the concentrations of the species involved in diffusion into the lesion. Furthermore, dissociation of the CPP - bound ACP will be facilitated by the acid generated during enamel remineralization. This would explain why the CPP – supported metastable calcium phosphate solutions are such efficient remineralizing solutions, since they would consume the acid generated during enamel lesion remineralization by generating more calcium and phosphate ions, including $CaHPO_4$, thus maintaining their high concentration gradients into the lesion. The remineralizing efficacy of f-TCP group was due to the presence of fluoride compatible functionalized calcium phosphate ingredient that imparts superior remineralization at both the enamel surface and within the subsurface lesions, thereby boosting the enamel surface strength. Although the Novamin dentifrice and f-TCP

dentifrice shows appreciable remineralizing potency, there was not much difference in the remineralizing capacity between these two groups. The remineralizing potential of Novamin group can be attributed due to the exchange of sodium ions from the Novamin particles with hydrogen cations (in the form of $H_3O^+$) in an aqueous environment of the tooth bringing about the release of calcium and phosphate ($PO_4^{3-}$) ions from the glass. A localized transient increase in pH occurs during the initial exposure of the material to water due to the release of sodium. This increase in pH helps to precipitate the extra calcium and phosphate ions provided by the Novamin to form a calcium phosphate layer. As these reactions continue, this layer crystallizes into carbonate – enriched hydroxyapatite (HCA). The combination of the residual Novamin particles and the newly formed HCA layer results in remineralization of the enamel surface and prevents further demineralization.

The results of the present study revealed that all the three remineralizing agents significantly increased the mineral content and micro hardness of enamel specimens after 15 days of treatment. After 30 days of treatment the increase in mineral content and micro hardness was still improved. Thus all the three remineralizing agents used showed promising remineralizing potential after 15 days and when the treatment period was increased to another 15 more days, it was found that the remineralizing potential was still increased. CPP - ACP group showed the maximum remineralizing potential followed by

CSP group and f-TCP group. It was thus concluded in the present study that all the three agents are equally effective in remineralizing the early carious lesions.

Within the limitations of the present in vitro study, it can be concluded that:

1. All the three remineralizing agents used in this study significantly increased the LAC and VHN values of the enamel specimens after a period of 15 days. The LAC and VHN values are still increased after another 15 days treatment with remineralizing agents

2. But when all the three remineralizing agents were compared, it was observed that CPP – ACP group was highly statistically significant, but there was not much statistically significant difference between groups II and III with respect to increase in mineral content and micro hardness of enamel following 30 days treatment.

# CONCLUSION

Within the limitations of the present study, it can be concluded that:

1. All the three remineralizing agents used in the study showed a significant remineralizing potential on demineralized enamel surfaces.

2. CPP - ACP group showed better remineralizing potential than the f-TCP group and the CSP group. Hence CPP - ACP can be considered as the materials of choice in remineralizing early enamel carious lesions.

3. f-TCP group and CSP group also shows promising remineralizing efficacy and hence they can also be considered as alternatives to CPP - ACP group in remineralizing early enamel carious lesions.

Further studies should be preferred with more sample size, various materials, time intervals and evaluation criteria to prove the efficacy in caries remineralization.

# SUMMARY

This study was done to compare the remineralizing potential of three different remineralizing agents on demineralized tooth surfaces using micro CT and micro hardness evaluation.

45 enamel specimens with buccal surface facing upwards were embedded in acrylic resin blocks and they were divided into 3 groups having 15 specimens in each group. Baseline evaluations of the samples were done following which the specimens were then demineralized using McInne's demineralizing solution in two cycles. After that remineralization procedure was done in two cycles using Casein phosphopeptide Amorphous calcium phosphate (CPP - ACP), 0.21% sodium fluoride - Tricalcium phosphate (f-TCP) and Calcium Sodium Phosphaosilicate (CSP) containing tooth pastes for groups I,II,III respectively. The specimens were evaluated for micro CT (Scanco$^{TM}$) and Vicker's Micro Hardness (Schimadzu$^{TM}$) testing at baseline, after demineralization cycle 2 and remineralization 1 and 2 cycles for determining the mineral concentration and the surface microhardness respectively. During the entire procedure the specimens were stored in artificial saliva.

In micro CT testing, specimens were scanned in horizontal thin sections at 100µm intervals and the digitalized images were captured by a computer at 1024x1024 pixels and the measurements were taken on three different locations and the mean of these

measurements were used as the Linear Attenuation Coefficient values of the specimen that was expressed in mg/cm3.

In microhardness testing, specimens were placed on the stage of tester, focused with 40x objective lens and a load of 100 g for 14 seconds was applied on the surface of specimen. The indentation formed was measured using the digital software and the average microhardness of the specimen was determined from five indentations and the values were expressed in VHN. The procedure was repeated for all the forty five specimens.

Data were analysed statistically using Statistical Package for Social Sciences. (SPSS) version - 10. Data were expressed in its mean and standard deviation and were analysed using One way ANOVA, paired-T test and Post-Hoc Duncan's Multiple Range test.

It was observed that all the three remineralizing agents used in the study significantly increased the LAC and VHN values of the enamel specimens following 15 days and 30 days application. But when the three agents were compared, it was observed that CPP - ACP (Group – 1) showed the better remineralizing potential than the other two agents and there is no statistical significance between f-TCP (Group - II) and CSP (Group – III).

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# INSTITUTIONAL ETHICS COMMITTEE AND REVIEW BOARD

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Thirurajapuram, Kavalkinaru Jn – 627 105, Tirunvelveli District.
(Constituted as per order no.RDC/CH/CR/5/2010)

DCI Recognition No : DE-3(44)-93/2246
Dated 09-11-1993

Affiliated to
The Tamil Nadu Dr. M.G.R. Medical University, Chennai



This ethical committee has undergone the research proposal submitted by **Dr. ARUN BALAKRISHNAN,** Post Graduate Student, Dept. of CONSERVATIVE DENTISTRY AND ENDODONTICS under the title **"COMPARITIVE EVALUATION OF THE REMINERALIZING POTENTIAL OF THREE DIFFERENT DENTIFRICES – AN IN VITRO STUDY EVALUATED USING MICRO CT AND MICROHARDNESS TESTING"** under the guidance of **Dr. R. JONATHAN, M.D.S** for consideration of approval to proceed with the study.

This committee has discussed about the materials being tested involved with the study, the qualification of the investigator, the present norms and recommendations from the Clinical Research Scientific Body and comes to a conclusion that this research protocol fulfills the specific requirements and the committee authorizes the proposal.

I.E.C. & I.R.B. RAJAS DENTAL COLLEGE & HOSPITAL ETHICAL COMMITTEE & REVIEW BOARD

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